"Immunohistochemical expression of BRCA mutations and its correlation with clinicopathological variables in Carcinoma Prostate"

Thesis

Submitted to the faculty of

All India Institute of Medical Sciences, Bhopal

For the award of the degree of Doctor of Medicine (MD) in the subject of

Pathology and Lab Medicine

By

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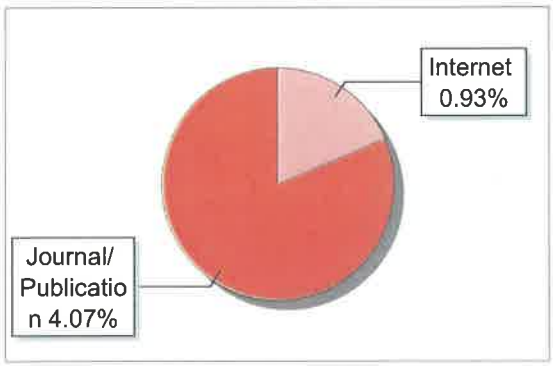

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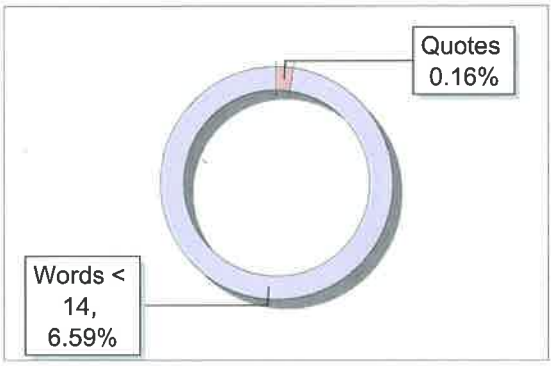

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TITLE

Immunohistochemical expression of BRCA mutations and its correlation with clinicopathological variables in Carcinoma Prostate.

INTRODUCTION

Prostate cancer is the second most frequent cancer and the fifth leading cause of cancer-related deaths among males globally in 2020(1).

GLOBOCON 2020 predicts that there will be 1.4 million new cases and 3,75,000 deaths related to prostate cancer. (2)

In a survey done by the American Cancer Society, 1,958,310 new cancer cases and 609,820 cancer deaths are predicted to occur in the United States in 2023(3)

In India, there have recently been 1,392,179 cases of cancer diagnosed; of those, 41,532 have been identified as prostate cancer, with a lifetime risk of 1 in 125 by the year 2020. (4)

The results from the recent data collected by the National Cancer Registry Program estimate that 4,88,970 cases will be diagnosed as prostate cancer in India by 2025. (5)

According to the geography, prostate cancer incidence rates range from 6.3 to 83.4 per 100,000 males, with the highest rates being recorded in Northern and Western Europe, the Caribbean, Australia/New Zealand, Northern America, and Southern Africa.

Like other malignancies, prostate cancer is more likely to develop and spread due to somatic or germline mutations. DNA damage response defects account for 25% of germline and somatic mutations in prostate cancer, with BRCA1 and BRCA2 mutations being the most common mutation to occur. (6)

Breast cancer susceptibility genes BRCA1 is located on chromosome 17q, and BRCA2 is located on chromosome 13q. They are both inherited via autosomal dominant with incomplete penetrance.

BRCA 1 and 2 are tumor suppressor genes involved in double-strand break repair, transcription, recognition, and other critical pathways for controlling DNA damage. (7) In BRCA1- and BRCA2-mutant cells, a deficiency in this DNA repair pathway drives the cells towards more error-prone repair pathways, leading to the accumulation of spontaneous and damage-induced chromosomal abnormalities and resulting in the development of tumors.

Breast, ovary, prostate, and other tissues like the pancreas and stomach all express BRCA 1 and 2 in a usual manner. When DNA is damaged, the BRCA1 gene causes cellular responses that can inhibit cell growth and promote apoptosis (8), and the BRCA2 gene is involved in DNA repair.

According to estimates, BRCA2 mutation carriers have a 20% lifetime risk of developing prostate cancer, but BRCA1 mutation carriers have a risk of 9.5% by age 65, comparable to non-carriers. (9)

Patients with genetic BRCA1 or BRCA2 mutations in prostate cancer present at younger ages, have more poorly differentiated tumours, and have more aggressive clinical disease progression. (10,11)Higher Gleason score, nodal involvement, metastatic disease at diagnosis, and T3/T4 stage are all associated with BRCA1 and BRCA2 mutations. (12)

BRCA1 mutations have been related to an increased risk of high-grade cancers such as breast, ovarian, and, more recently, prostate. (13,14)

Numerous studies have shown that BRCA2 mutations enhance the risk of prostate cancer by roughly 3- to 8.6-fold. BRCA1 mutation can practically quadruple the risk. Men and women with pancreatic cancer and men with prostate cancer were considerably more likely to have a BRCA2 mutation. (15,16)

Germline mutation status is becoming a more crucial factor in management options due to the reasonably consistent associations between BRCA2 mutations and earlier-onset, more aggressive/higher-grade prostate cancers with worse outcomes.

Ki 67 is a well-known indicator of a poor prognosis and therapeutic resistance in prostate cancer. It is regarded as a key indicator of distant metastases and overall mortality. (17)

Prostate cancer continues to be a significant health problem despite recent advances in treatment. Androgen deprivation therapy (ADT), combined with chemotherapy drugs, is the first-line treatment for metastatic prostate cancer. However, most patients eventually acquire castration-resistant prostate cancer, a resistant disease. New clinical targets are, therefore, crucial.

Like many other malignancies, prostate cancer develops and spreads mainly due to angiogenesis. Matrix metalloproteinases (MMPs), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and cyclooxygenase-2 (COX-2) have been released by prostate cancer. It has been established that Micro-vessel density (MVD), a measurement of Prostate cancer

angiogenesis, can be used to predict metastasis and overall survival. The future study of anti-angiogenic therapy in metastatic castration-resistant prostate cancer is still promising. (18)

Studies have revealed that PARP inhibitors could halt the progression of localised prostate cancer to castration-resistant prostate cancer, resulting in a decreased expansion of castration resistance. Additionally, mounting evidence shows that patients with advanced prostate cancer with inherited mutations in the BRCA or other DNA repair genes respond well to PARP inhibitors and platinum-based chemotherapy.

The use of PARP inhibitors has significantly advanced the treatment of prostate cancer. More clinical trials are being conducted to evaluate their effectiveness in this area. If they could target a larger patient group, it would significantly lower the morbidity and mortality rates associated with prostate cancer.

This study has emphasized the IHC expression of BRCA mutations and its correlation with clinicopathological variables to determine prognosis in Carcinoma Prostate

REVIEW OF LITERATURE:

Prostate cancer is the second most common cancer and ranked fifth in causing cancer-related deaths in men worldwide in 2020.

Inherited mutations in BRCA genes are associated with an increased risk of developing breast, ovarian, prostate, and other cancers.

PROSTATE CANCER

Etiology:

Prostate cancer is a complex, multivariate etiology with modifiable and unmodifiable risk factors. Age, positive family history, and African ancestry are a few of the most significant risk factors. (19)

Epidemiological research suggests that nutrition, lifestyle, and environmental factors can affect the risk of prostate cancer and the disease's course.

Age:

50% of males between the ages of 70 and 80 had a chance to get a histological diagnosis of prostate cancer (20)

Family history :

Having a family history of breast cancer increased the risk of prostate cancer by 21% overall, having a family history of prostate cancer alone increased risk by 68%, and having a family history of both cancers also increased risk, according to a significant prospective study conducted in the United States involving 37002 men over a study period of 16 years. One possible explanation for the link between family breast cancer and a higher risk of prostate cancer is the BRCA gene mutation. (21)

Ethnicity:

Men of black African heritage have higher incidence, severity, and mortality rates. (19)

In 48 nations, including several in Sub-Saharan Africa, The Caribbean, Central and South America, prostate carcinoma is the most common cancer among males and the leading cause of cancer mortality. (2)

Smoking and Alcohol consumption:

Recent research shows prostate cancer incidence and mortality correlate with smoking and alcohol consumption.

According to Huncharek et al.'s (2010) meta-analysis of 24 cohort studies with 21,570 prostate cancer patients, current heavy smokers had a 24% to 30% higher chance of dying from prostate cancer than nonsmokers. (22)

Alcohol consumption and the risk of prostate cancer have a significant dose-response association, according to a systematic review and meta-analysis. (23)

Obesity :

According to a 2011 study by Cao Y. and Ma et al., an increase in BMI of 5 kg/m2 was linked to a 20% increase in prostate cancer-specific mortality and a 21% increase in the probability of recurrence. (24)

IGF-1 (Insulin-like growth factor-1), sex hormones, and adipokines (particularly adiponectin) are some of the underlying potential causes.

Because of its role in the anti-proliferative, anti-inflammatory, AMPK, and Wnt signalling pathways, Liao et al. (2011) reported that adiponectin had an inverse relationship with prostate cancer risk, i.e., the greater the risk of prostate cancer, the lower the concentration of adiponectin. (25)

As a result, adiponectin may one day serve as a biomarker for the detection of prostate cancer.

Metabolic syndrome:

The presence of three or more of the following factors indicates the presence of the metabolic syndrome: 1) Central obesity, 2) Diabetes (raised fasting blood sugar), 3) High blood pressure, 4) Increased triglyceride levels, 5) Lower levels of high-density lipoprotein (HDL)

The results of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) study in 6426 men showed that having metabolic syndrome components significantly increased the probability of developing higher-grade prostate cancer. (26)

Other factors:

Physical activity (Exercise) :

A large observational research of 2705 men with prostate cancer found that men who engaged in at least three hours of vigorous exercise per week had a 61% lower risk of prostate cancer-specific death than those who engaged in less than one hour per week. (27)

Diet: Consuming more fatty, westernised, and processed meals raises the risk, whereas eating more fish and lycopene-rich foods (tomatoes, watermelons) lowers the risk of prostate cancer. Prostate cancer risk is increased to some extent by vitamin D deficiency as well. (28)

Medications: Prostate cancer risk is decreased in patients receiving treatment with alpha-reductase inhibitors, Metformin, statins, and aspirin. (29)

PATHOPHYSIOLOGY OF PROSTATE CARCINOMA

Anatomy and embryological development :

The prostate is a male sex glandular structure that develops embryologically from the urogenital sinus, which is a caudal extension of the hindgut.

At the embryonic cloaca, the urogenital sinus and hindgut are initially fused to form a single excretory tract. The cloaca later divides into separate urogenital and anorectal channels at eight weeks of gestation.

The primitive urogenital sinus is then subdivided into the bladder at its rostral end, the urogenital sinus (UGS) in the middle, and the penile urethra at the caudal end.

The prostate is formed at about 10 weeks of gestation through epithelial budding from the urogenital sinus, which depends on androgen receptor activity. (30)

Anatomical zones of the prostate:

Under the bladder in front of the rectum is where the prostate is situated. The prostate is anatomically separated into three zones. (31)

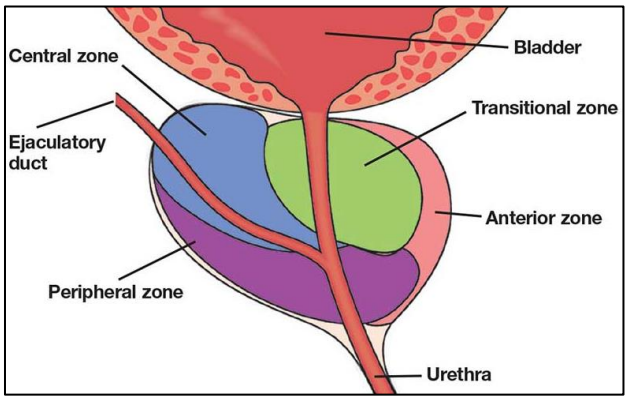


Figure 1. Anatomical Zones of prostate (32)

1) Central zone (20%)- Ejaculatory duct is surrounded by the central zone (20%).

2) Transition zone (10%) - The most typical location for benign prostatic hyperplasia (BPH), it surrounds the urethra

3) The peripheral zone (70%) is the most common site for malignancy.

Functions:

One of the prostate's primary functions is to provide semen with nutrient-rich alkaline fluid, and the other is maintaining the pH of semen (33)

Due to its strong dependency on androgenic hormone (testosterone) signalling and other embryological signalling pathways, including Sonic Hedgehog activation and improper expression of the Gli-1 oncogene, prostate cancer is the most prevalent malignancy than any other cancer of urogenital organs. (34)

Normal Histology of prostate gland:

Prostatic epithelium and stroma make up the prostatic gland.

There are three different cell types in the prostatic epithelium.

1) Luminal cells: Prostatic fluid and PSA are secreted by luminal cells, which express androgen receptors (ARs).

2) Basal cells: Generating proteins necessary for the development of the basement membrane and fluid generation

3) Neuroendocrine cells: Uncommon cells scattered across basal and luminal cells.

A basal to luminal cell ratio of 1:1 in the prostate is considered typical.

The fibromuscular stroma, a unique stroma that is characteristic of the prostate, is created when smooth muscle cells and fibroblasts combine.

HISTOPATHOLOGICAL FEATURES OF PROSTATE ADENOCARCINOMA

Most of the prostate cancers are Acinar adenocarcinomas (90-95%) followed by secondary bladder transitional cell carcinoma(5%) and prostate sarcoma (1.25%) that arise from the peripheral prostatic gland. (35)

When compared to basal cell tumours, luminal cell tumours are more aggressive. (36)

The following entities make histological diagnosis of prostate cancer:

1) Loss of surrounding basal cells

2) Damage to the basement membrane between the epithelium and the stroma

3) Loss of the typical glandular architecture

4) Luminal cell nuclear atypia (37)

Clinical Features:

As the prostate is a urogenital organ, patients presenting complaints are lower urinary tract symptoms such as reduced urine stream, urgency, nocturia, hematuria hesitance, and difficulty in urinating. When a patient has an advanced stage disease, they may exhibit pathological fractures, bone pain, and, very occasionally, neurological signs.

Prostate cancer screening and current diagnostics:

1) Digital rectal examination (DRE)

2) Markers in serum and urine

3) Radiological Imaging

4) Tissue Diagnosis

Digital rectal examination: Since decades ago, during the pre-MRI period, the digital rectal exam (DRE) has been a standard component in the diagnosis of prostate cancer. Additionally, it aids in risk evaluation and prognostication.

According to research by Cupp et al., the DRE's sensitivity ranges from 69% to 89% and its specificity from 84% to 98%. A positive predictive value between 25% and 35%. (38)

If located in the posterior peripheral zone, it can identify prostate cancer with a volume of >0.2 ml and can be utilized to raise suspicion without regard to PSA.

Serum markers:

Role of Prostate Specific Antigen (PSA) in Prostate cancer:

In general, PSA is a serine protease belonging to the kallikrein family produced by the prostate's secretory epithelium in both benign and malignant conditions. (39)

Age-specific reference ranges are helpful because the serum PSA concentration correlates directly with patient age.

Oesterling et al.'s community-based investigation found the (95th percentile) reference range for serum PSA in men is 0.0 to 2.5 ng/mL for those aged 40 to 49, 0.0 to 3.5 ng/mL for those aged 50 to 59, 0.0 to 4.5 ng/mL for those aged 60 to 69; and 0.0 to 6.5 ng/mL for those aged 70 to 79 (40)

For prostate cancer screening, diagnosis, treatment, and prognosis, serum PSA levels have been employed. (41,42)

Derivatives of Prostate Specific Antigen (PSA): 1) PSA Density 2) PSA Kinetics

PSA Density: Total PSA divided by prostate volume can be used to calculate it. Prostate cancer risk is correlated with an elevated PSA density. The PSA density cutoff ranges from 0.12 to 0.15 ng/ml/cc. (43)

PSA Kinetics: Recent studies have shown that PSA velocity (change in PSA over time, measured in ng/ml/year), as well as PSA doubling time (the number of months for the PSA to increase two-fold), are currently ineffective in the diagnosis of prostate cancer.

As per the advanced trend, additional serum assays are currently performed to enhance diagnostic prediction and decrease the number of unnecessary biopsies. (44)

The following additional serum assays are available commercially: **Four kallikrein score and the prostate health index.**

The Prostate health index (Phi) uses a formula to combine the results of total PSA, free PSA and [-2]proPSA

Four kallikrein score combines patient age, DRE results, and prior biopsy status with four kallikrein indicators (Free PSA, total PSA, intact PSA, and kallikrein-like peptidase 2 [hK2]).

Urine markers:

Prostate cancer gene 3 (PCA3)

Using quantitative real-time PCR, this prostate-specific non-coding mRNA may be detected in urine that has been collected following prostatic massage and is overexpressed in prostate cancer.

In patients who have had a benign biopsy, PCA3 may be particularly helpful in predicting the presence of prostate cancer. (45)

It is unaffected by prostate size, prostatitis, or treatment with alpha-reductase inhibitors, in contrast to PSA. (46,47)

Transmembrane protease serine 2 gene (TMPRSS2): ERG Fusion

ERG, an ETS family transcriptor gene, fuses with the transmembrane protease serine 2 gene (TMPRSS2) to cause overexpression of the gene in prostate cancer.

78 men with prostate cancer-positive biopsies and 30 men with prostate cancer-negative biopsies participated in an experimental study conducted by Hessels et al. For the detection of TMPRSS2-

ERG fusion transcripts in urine sediments, the researchers used semiquantitative reverse transcription-PCR (RT-PCR) analysis followed by Southern blot hybridization with a radiolabeled probe. The study achieved a sensitivity of 37% and a specificity of 93%.(48)

Additional research demonstrated that combining PCA3 and TMPRSS2: ERG fusion improves diagnostic performance.

ROLE OF IMAGING IN PROSTATE CANCER DIAGNOSIS:

Imaging is becoming increasingly important in diagnosing prostate cancer and can be utilised to detect clinically relevant prostate carcinoma.

Transrectal Ultrasound (TRUS):

As technology advanced over time, traditional B-mode ultrasound evolved into colour Doppler ultrasound, contrast-enhanced ultrasound, and, ultimately, sonoelastography.

Based on the idea that the elastic characteristics of benign and malignant prostate tissue differ significantly, sonoelastography measures the response of tissues to harmonic mechanical excitation and uses Doppler ultrasound to find areas of aberrant stiffness. (49)

Recently, Multiparametric US (mpUS) increased the sensitivity for detecting clinically significant prostate cancer to 74% by combining all the entities mentioned above. (50)

However, it has limitations in determining tumor burden and extraprostatic extension. TRUS-guided biopsy has dramatically boosted the detection rate of prostate cancer.

Magnetic Resonance Imaging (MRI)

The size and extent of prostate malignancies can be determined using MRI, a non-invasive imaging technique. (51)

The additional benefit of MRI is that it can be used to assess prostate cancer patients, who are more likely to develop bone involvement.

A reliable method for identifying prostate cancer with high sensitivity and specificity is Multiparametric MRI (mp MRI). Mp MRI uses both functional and conventional MRI, including dynamic contrast enhanced (DCE) imaging and diffusion weighted imaging (DWI). (52)

Prostate Imaging-Reporting and Data System (PI-RADS)

The Prostate Imaging-Reporting and Data System (PI-RADS), similar to BIRADS and TIRADS, offers a standardised method of reporting each lesion by assigning a score between 1 and 5 (the higher the score, the greater the probability that the lesion is clinically significant prostate cancer).

A PIRADS score of 1 or 2 suggests highly unlikely to be cancer; PIRADS 3 is equivocal; PIRADS 4 or 5 is highly suspicious for clinically significant prostate cancer (Gleason 3+4=7 and higher).

It is advised to do a biopsy and obtain histological confirmation for all PIRADS 3, 4, and 5 lesions. (53)

Bone Scintigraphy (Bone Scanning)

Technetium Tc 99m methylene disphophonate (Tc 99m MDP)-based bone scanning is the most accurate way to detect bone metastases in men with prostate cancer. (54,55)

Bone scans are advised by the NCCN's oncology guidelines for high-risk patients with PSA > 20 ng/ml, Gleason score > 8, or pT3 cancer. (56)

In addition to allowing for more significant morphological and anatomical characterisation, the combined use of single photon emission computed tomography (PET-CT) and bone scan substantially improved diagnostic accuracy. (57)

METHODS OF TISSUE DIAGNOSIS

1) Prostate Biopsy:

The current practice is to take a series of core biopsies from suspicious lesions, guided by ultrasound, starting with six and then eventually twelve guided biopsies from six different sites, including the apex, middle, and base of each lobe. (58)

The most common approaches for doing prostate biopsies are transrectal and transperineal. A meta-analysis conducted in 2019 by Xiang et al. suggests that both of these methods produce nearly identical outcomes. The most frequent way is transrectal, but transperineal has a lower risk of fever and rectal bleeding. (59)

Prebiopsy multiparametric MRI (mp-MRI) is currently the gold standard for targeted biopsies.

According to a Cochrane database study that compared the two techniques, mp-MRI increases the number of significant cancers diagnosed compared to systematic biopsy. (60)

Fusion biopsy, Cognitive targeted biopsy, and Direct in-bore targeted biopsy are three different techniques to perform targeted biopsies of lesions found on mp-MRI.

Transurethral Resection of Prostate (TURP) :

To make sure that the percentage of specimen area with malignancy is representative of the entire submission, TURP chips are randomly submitted.

Transurethral resection specimens weighing 12 grams or less should be submitted completely, while specimens weighing more than 12 g should be submitted in two parts: the initial 12 g plus one cassette for every additional 5 g of remaining tissue. (61)

If an unanticipated carcinoma is discovered in tissue submitted, especially in younger patients, and it involves 5% or less of the tissue analysed, the remaining tissue may be submitted for microscopic examination. (62)

Radical Prostatectomy:

To evaluate the patient's prognosis and treatment, a thorough, proper examination of the radical prostatectomy is necessary for the detection of tumour features such as grade, pathological staging, extraprostatic extension, surgical margin status, etc. (21)

MACROSCOPIC/GROSS EXAMINATION :

The grossly recognisable tumours are firm, distinct, tan white and yellow-colored.

MICROSCOPIC EXAMINATION/HISTOPATHOLOGY:

The architectural pattern of the tumor and tumor cells with nuclear features, cytoplasmic features, and intraluminal contents are used to diagnose prostate adenocarcinoma.

Nuclear features:

1. Prominent nucleoli
2. Nuclear hyperchromasia
3. Nuclear enlargement
4. Mitotic figures
5. Apoptotic bodies

Cytoplasmic features:

1. Amphophilic cytoplasm
2. Sharp luminal border

Intraluminal contents:

1. Blue-tinged mucinous secretions

2. Crystalloids
3. Pink amorphous secretions

GRADING BASED ON ARCHITECTURAL PATTERN: GLEASON GRADING

Dr Donald Gleason, a pathologist, created the Gleason prostate grading system in the 1960s, which has since become extensively used worldwide. A study of 270 patients from the Minneapolis Veterans Administration Hospital served as the basis for the very initial description of this method. (63)

Changes/Modifications in the Gleason grading:

Gleason originally planned to categorise carcinomas into four patterns; pattern 3 includes the cribriform and individual cells.

In 1974, the study's participant population was increased to 1032 cases. Gleason pattern 4 was defined as a "raggedly infiltrating, fused-glandular tumour, frequently with pale cells, may resemble hypernephroma of the kidney."

In 1977, when Mullinger further refined the Gleason classification, The Gleason pattern 3 was defined as having a "smooth and typically rounded edge" under which papillary and cribriform tumour was classified.

According to a significant editorial from 2000, a Gleason score of 2–4 should not be assigned to cancer on needle biopsy.

The Gleason scoring system evolved as a result of improvements in prostate cancer diagnosis and visualisation techniques. Changes were made initially in 2005 and, more recently, in 2014. (64)

The International Society of Urologic Pathology (ISUP) had two big consensus meetings in 2005 and 2014, which resulted in a considerable modification of the Gleason grading system for prostate cancer from its original 1960s–1970s scheme.

The purpose of both meetings was to reach an agreement among top urological pathologists on particular Gleason grading topics, including those in which there needed to be more data or more information.

The Gleason grading system only considers the architectural pattern of the tumour, and a score is calculated as the sum of the two most common grade patterns.

The x4 and x10 objectives are used to perform the Gleason grading; if necessary, the x20 objective can be utilised to confirm the grading. When there is just one histological pattern present in a tumour, both the primary and secondary patterns are given the same number (for example, 3+3=6). However, a tumour is assigned a score of 7 (3+4=7) if it predominantly exhibits Gleason pattern 3 and less Gleason pattern 4. (63)

If each core is received separately and is labelled, scores should be given for each core individually.

The lower grade patterns in core biopsy or radical prostatectomy should be ignored if they account for less than 5% of the tumour. (65)

Because they are not very reproducible, the Gleason scores between 2 and 5 are no longer employed. Poor correlation between radical prostatectomy grade and patient's tumour, giving physicians the impression that the patient has a harmless indolent tumour. (66)

Gleason pattern 3:

The most typical pattern has well-formed, distinct, relatively uniform glands infiltrating between glands; the glands may be compressed or angulated and spaced more than one gland diameter apart.

The glands may be of various sizes, from small, medium and large glands with lumina, but glands still separate.

Even though the glands are identical in size to normal glands, the cribriform pattern, which was formerly in the Gleason 3 pattern, is no longer considered to be Gleason 3.

Gleason pattern 4:

This pattern consists of clusters of poorly defined, ill-formed, or fused glands.

According to the 2014 WHO/ISUP grading consensus conference, the cribriform pattern, that frequently merges with the papillary structure and should not have any stroma or mucin in between the individual or fused glandular structures, is also designated Gleason pattern 4 (67)

A small cribriform variety known as the Glomeruloid pattern (2014 consensus) has a tuft of cells largely disconnected from the duct space around it, except for one point of attachment. (68)

Small, hyperchromatic nuclei, nests of clear cells that resemble renal cell carcinoma, the fusion of acini into more solid sheets, and the appearance of back-to-back glands without stroma between them are all characteristics of the Hypernephroid pattern.

Gleason pattern 5:

Sheets of tumor cells, individual tumor cells that possibly form cords, linear arrays, and solid tumor nests make up this pattern. (69)

Comedonecrosis: Pattern 5 also refers to central necrosis with intraluminal necrotic cells or karyorrhexis within papillary or cribriform spaces.

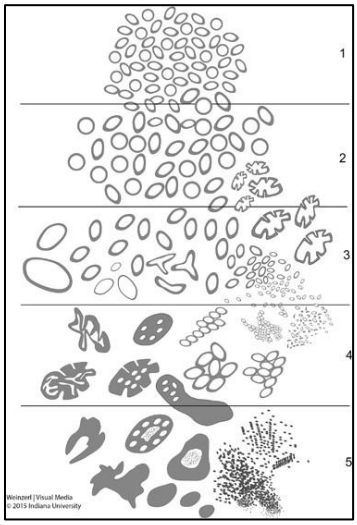


Figure 2: Modified Gleason grading schematic diagram, according to International Society of Urological pathology (ISUP 2015) (70)

NEW RECOMMENDATION: Reporting percentage of Pattern 4:

Reporting percent pattern 4 with a Gleason score of 7 or more in both needle biopsies and Radical prostatectomy samples was a significant new suggestion that emerged from the 2014 Consensus Conference and was later supported by the 2016 WHO. (37)

As there are different radiation therapy treatments for Gleason scores 3+4=7 (Grade Group 2) and 4+3=7 (Grade Group 3), recording the percent pattern 4 could also be beneficial in cases that are on the borderline of Gleason score 3+4=7 versus Gleason score 4+3=7. (71)

New Contemporary Prostate Cancer Grading System

The Gleason prostate cancer (PCa) grading system still has significant flaws despite updates in 2005 and 2014 (72)

The advantages of the New Contemporary Prostate Cancer Grading System include:

1) Grade stratification that is more precise than the Gleason system in use today

2) A simplified 5-point grading system as opposed to multiple possible scores based on different Gleason pattern combinations

3) The lowest grade is 1, as opposed to the standard Gleason score of 6, which may help to prevent overtreating men with indolent prostate cancer.

The New Contemporary Prostate Cancer Grading System was proposed in a 2015 study by Jonathan et al. in 20,845 patients diagnosed with localised prostate cancer. (72)

Table: Grade Groups

Grade Group	Gleason Score	Definition
1	Less than or equal to 6	Only individual discrete well-formed glands
2	3+4=7	Predominantly well-formed glands with lesser component of poorly formed/fused/cribriform glands
3	4+3=7	Predominantly poorly formed/fused/cribriform glands with lesser component (#) of well-formed glands
4	4+4=8	Only poorly formed/fused/cribriform glands
	3+5=8	Predominantly well-formed glands and lesser component (##) lacking glands (or with necrosis)
	5+3=8	Predominantly lacking glands (or with necrosis) and lesser component (##) of well-formed glands
5	9-10	Lack gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands (#)

#For cases with greater than 95% poorly formed/fused/cribriform glands on a core or at radical prostatectomy, the component of less than 5% well-formed glands is not factored into the grade; should therefore be graded as grade group 4.
##Poorly formed/fused/cribriform glands can be a more minor component.

Figure 3: Depicting the New Contemporary Prostate Cancer Grading System (73)

TNM Classification of carcinoma of prostate (74)

Primary Tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically inapparent tumor not palpable not visible by imaging
T1a	Incidental tumor in < 5% of TUR tissue
T1b	Incidental tumor in > 5% of TUR tissue
T1c	Needle biopsy prompted by elevated PSA
T2	Organ confined
T3	Tumor extends beyond the prostatic capsule

T3a	Extracapsular, unilateral and bilateral or microscopic invasion of bladder neck
T3b	Tumor invades seminal vesicles (s)
T4	Tumor invades external sphincter, rectum, pelvic side wall
Lymph Nodes	
Nx	Regional nodes were not assessed
N0	No regional (below level of bifurcation of common iliac arteries) nodes
N1	Regional node metastases – including pelvic, hypogastric, obturator, iliac, sacral
Distant Metastases	
Mx	Regional nodes not assessed
M0	No Metastasis
M1	No distant metastasis

M1a	Non-regional lymph nodes (outside true pelvis)
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

Table 1: Table depicting TNM Staging of Prostate Carcinoma

TUMOR SPREAD :

The prostatic carcinoma spreads through lymph nodes and blood vessels in addition to infiltrating tissues nearby.

Direct tumours spread to the seminal vesicles and periprostatic fat, whereas advanced tumours spread to the pelvic soft tissues, bladder, and rectum.

Metastatic spread to regional lymph nodes more commonly to iliac, obturator and presacral lymph nodes (75)

Involvement of regional lymph nodes is observed in 5-12% of patients. (76)

Distant spread includes cancer affecting the bones, liver, thorax, and digestive system.

Prostatic acinar adenocarcinoma variants:

Atrophic variant:

Described as malignant glands with reduced cytoplasm, observed in 2-16% of adenocarcinomas.

The atrophic pattern is frequently Gleason pattern 3 and has an infiltrative growth pattern. It is made up of single and distinct glands. (77)

Pseudohyperplastic variant:

Some architectural growth patterns in this variant are branching luminal undulations and papillary infoldings. Gleason pattern 3 has been assigned to pseudohyperplastic adenocarcinoma. (78)

Microcystic variant:

Acinar adenocarcinoma of intermediate gland size having cystic dilatation is designated Gleason pattern 3

Foamy gland variant:

Histologically, the foamy gland is characterized by abundant foamy cytoplasm with a non-foamy gland carcinoma component. Gleason's score of 3+3=6 would be attributed to the majority of cases of foamy gland cancer.

Mucinous (colloid) variant:

The tumour is made up of individual, fused glands cribriform nests floating within a mucinous matrix, allocated a score of 4+4=8 (79)

Signet ring-like cell variant:

At least 25% of the tumour must have signet ring-like cells for a diagnosis to be made. These cells lack intracellular mucin and contain empty vacuoles. Vacuoles are frequently observed in Gleason patterns 4 and 5.

Pleomorphic giant cell variant:

This variation includes prostate adenocarcinomas with giant bizarre anaplastic cells that lack the spindle cell component and have pleomorphic nuclei.

Sarcomatoid variant:

Sarcomatoid carcinoma is a biphasic malignant tumour with differentiation of the mesenchyme and epithelium. (80)

Immunohistochemical diagnosis of Prostate adenocarcinoma:

Adenocarcinomas that are invasive lack basal cells. Antibodies used in immunohistochemistry target basal cells. Antibodies that react with cytokeratins with high molecular weight, such as p63 and p40. (81)

Alpha Methylacyl CoA Racemose (AMACR) is a marker that often exhibits granular cytoplasmic staining and is expressed in most cases of high-grade prostatic intraepithelial neoplasia (PIN) and prostate cancer. (82)

Prostatic Intraepithelial Neoplasia (PIN)

Low grade PIN:

Darker and more complex than normal glands are visible at scanning magnification

Architecture displaying epithelial cell crowding, pseudostratification with irregular spacing with an Intact basal layer

The nuclei are enlarged with increased variability in nuclear size and nuclear hyperchromasia, indistinct and rare nucleoli.

High grade PIN:

Prominently visible nucleoli at 200x or lower magnification is a diagnostic sign.

Medium to large ducts and acini with enlarged hyperchromatic nuclei and amphophilic cytoplasm; however, the enlargement is less than in low grade PIN

Common architectural patterns:

• Flat: 1 - 2 layers of simple epithelium

• Tufted: Stratified epithelium with small luminal protrusions

• Micropapillary: Filliform structures lacking a true fibrovascular core

• Cribriform: Epithelial proliferation with punched-out spaces

Signet ring cell, foamy gland, mucinous, inverted, and small cell neuroendocrine patterns are some of the less frequent patterns.

RELATION BETWEEN BRCA MUTATAIONS AND PROSTATE CARCINOMA:

BRCA mutations in Prostate cancer

The Breast Cancer Linkage Consortium reported in 1999 that the prevalence of prostate cancer was higher among relatives with an increased incidence of breast and ovarian cancers, providing the first evidence of a connection between BRCA germline mutations and prostate cancer. BRCA1 and BRCA2 mutation carriers had a relative risk of prostate cancer that was 1.8 times higher and 7–23 times higher, respectively, than the general population. (83)

Van Asperen et al. (2005) Reported that BRCA2 mutation carriers had higher risks for various other malignancies in addition to breast and ovarian cancer, according to a nationwide study conducted in the Netherlands by Van Asperen et al. & his colleagues. Prostate, pancreatic, bone, pharynx, and a few cases of digestive system cancer are among the other cancers. The study involved 139 BRCA2 carriers (both males and females), and interestingly, the findings indicate that men are more likely than women to develop cancer, especially when they are younger. This

study highlights the importance of screening for BRCA mutations in families and the higher risk of prostate cancer in BRCA2 mutation carriers. (84)

Tryggvadottir et al. (2007) Identified 596 cases of prostate cancer in Iceland between 1955 and 2004; of them, 527 cases had BRCA mutation status identified. 30 cases have been shown to be mutation carriers, while the remaining cases are not. BRCA2 999del5 mutation carriers had advanced tumour stage (stages 3 or 4; 79.3% versus 38.6%; P .001), younger age at diagnosis (69.0 versus 74.0 years; P =.002), and high tumour grade (grades G3-G4; 84.0% versus 52.7%; P =.007), with this study concluding that BRCA2 mutation carriers are more frequently associated with short median survival time, poor prognosis and fatal prostate cancer (85)

Moro et al. (2008): According to Moro et al., a functional BRCA2 gene may restrict the ability of cancer cells to spread by suppressing the enzyme matrix metalloproteinase 9 (MMP9) through the inhibition of pi3-kinase/AKT and the activation of MAP/ERK, which blocks cancer cell invasion and migration. The study, which involved 80 men with prostate adenocarcinomas, showed that cancer cells lose the BRCA2 protein compared to normal and hyperplastic prostate epithelium. (86)

Narod et al. (2008): The survival of males with prostate cancer who had a BRCA1 mutation was compared to that of men who had a BRCA2 mutation by Narod et al. and his colleagues. As a result, BRCA1 had a median survival time from diagnosis of 8.0 years compared to BRCA2's 4.0 years. This study involved 119 individuals with BRCA1 mutation, and 182 patients with BRCA2

mutation had prostate cancer. They discovered cases from BRCA2 families had a 70% higher risk of dying than those from BRCA1 families. This research prioritizes the need for targeted chemotherapy for males with the BRCA2 mutation diagnosed with prostate cancer. (87)

Edwards et al. (2010): In a cohort study of 263 prostate cancer patients, Edwards et al. compared two cohorts: one with young-onset prostate cancer (6 of 263 cases had a germline BRAC2 mutation), and the other with prostate cancer patients with known BRCA2 mutation carriers (15 cases).

Kaplan-Meier estimates of survival data were compared with a control series of patients in a single clinic. In all cases with a germline BRCA2 mutation, the median survival was shorter at 4.8 years than in controls at 8.5 years (P=0.002), suggesting that prostate cancer in BRCA 2 mutation carriers had a poor prognosis. (11)

Gallagher et al.(2010): 26 cases of prostate cancer having the Ashkenazi founder mutations BRCA2 6174delt (20 males) and BRCA1 185delag (6 men) were reported by Gallagher et al. in 2010 along with 806 non-carriers. Poorly differentiated tumours were more common in patients with BRCA2 6174delt than in non-carriers. (85% vs. 57%). This study found that individuals with BRCA2 mutations had a higher histologic grade, an aggressive clinical course, and a higher risk of prostate cancer. (88)

Kote-Jarai Z et al. (2011): The BRCA gene was analysed in 1832 cases of prostate cancer by Kote-Jarai Z et al.

in 2011. A unique high-throughput multiplex fluorescence heteroduplex detection technique was used to identify the BRCA2 gene. This method found 19 protein-truncating mutations, of which 16 were frameshift mutations, and 3 were nonsense mutations. Nine patients had a family history of breast or ovarian cancer among first- or second-degree relatives (47.4%), and all 19 detrimental mutation carriers developed the disease at less than or equal to 65 years of age. These findings suggested that systematic testing for germline BRCA2 mutations in early-onset Prostate cancer cases will aid in defining the prevalence and risk of BRCA2 mutations and may help direct the treatment approaches. (89)

Thorne et al. (2011) Reported an investigation of the prognosis of prostate cancer in 40 individuals who carried the BRCA2 mutation compared to 97 patients with BRCA wild-type. Mutations in BRCA2 had a poorer CSS. It has been demonstrated that the BRCA2 mutation status is a reliable predictor of CSS. (90)

Castro et al. (2013):

They examined the tumour characteristics and outcomes of 2,019 prostate cancer patients. The study group comprised 18 BRCA1 carriers, 61 BRCA2 carriers, and 1,940 noncarriers. The relationships between BRCA1/2 status and other prostate cancer prognostic variables were assessed using the Kaplan-Meier method and Cox regression analysis. The cases with germline BRCA1/2 mutations were more frequently associated with Gleason 8 (P =.00003), T3/T4 stage (P =.003), nodal involvement (P =.00005), and metastases at diagnosis (P =.005) than in noncarriers, demonstrating the significance of BRCA mutations screening and highlighting the need for clinical management of these patients to be tailored to these patients' needs. (12)

OTHER NOVEL GENETIC MUTATIONS AND PATHWAYS IN PROSTATE CANCER

In 2015, the Cancer Genome Atlas (TCGA) examined 333 primary prostate cancer specimens and observed molecular heterogeneity. 19% of localised prostate tumours had inactivated DDR genes, the bulk of which were located in the HR pathway and included BRCA1 (1%), BRCA2 (3%), and RAD51C (3%). In addition, the following vital kinases were deactivated: CDK12 (2%), ATM (4%), and FANCD2 (7%) (91)

In a 2016 study by the International Stand Up to Cancer/Prostate Cancer Foundation team, 150 metastatic prostate cancer tissues were examined, and it was found that 23% had somatic DDR changes and 8% had germline DDR mutations. The samples with the highest number of mutations were BRCA2 (13%) and ATM (7.3%), followed by MSH2 (2%) and BRCA1, FANCA, MLH1, RAD51B, and RAD51C (0.3% for all).

Schoenborn et al. (2013): Known aberrant genetic processes in prostate cancer include somatic copy number alterations, point mutations, chromosomal number alterations, and structural alterations. In over 90% of PCa cases, somatic copy number alterations can be discovered. Primary PCa frequently shows deletions on various chromosome numbers, such as 6q, 8p, 10q, and 13q, as well as other particular genes, such as NKX3-1, PTEN, BRCA2, and RB1. (92)

Pritchard et al. (2016): A cohort of 692 patients with metastatic prostate cancer, including 82 men (11.8%), had mutations in 16 DNA repair genes, according to Pritchard et al. BRCA2 (5.35%), ATM (1.6%), CHEK2 (1.9%), BRCA1 (0.9%), and PALB2 (0.4%) were the most frequently affected genes by mutations. (93)

Nicolosi et al. (2019): Between 2013 and 2018, 3,607 males with a history of prostate cancer were studied for germline mutations by Nicolosi et al. They discovered that 620 men (17.2%) had germline mutations, only 30.7% of which were BRCA1/2 mutations; other mutations found included ATM, PALB2, CHEK2, and mismatch repair genes PMS2, MLH1/2/6. (94)

BRCA Mutations in other cancers:

Men and women with pancreatic cancer and men with prostate cancer were considerably more likely to have a BRCA2 mutation. (95)

Mutations in the BRCA1 and BRCA2 genes are notably associated with inherited breast and ovarian cancers, and nearly 30-40% of sporadic malignancies are associated with loss of BRCA1 expression. The lifetime risk of breast cancer in BRCA1 and BRCA2 mutation carriers is 45–80%, and for ovarian cancer is 45–60% and 11–35% for BRCA1- and BRCA2 -mutation carriers, respectively.

AIM:

To evaluate immunohistochemical expression of BRCA1 and BRCA2 mutations and its correlation with clinicopathological variables to determine prognosis in prostate adenocarcinoma.

OBJECTIVES

1. To perform IHC for BRCA1 and BRCA2 in paraffin-embedded prostate biopsy specimens of patients diagnosed with prostate adenocarcinoma.
2. To determine the prevalence of BRCA 1 and 2 in prostate adenocarcinoma.
3. To determine the association of BRCA1 and BRCA2 expression with clinicopathological factors (age of patient, serum PSA level, Gleason score/New contemporary prostate cancer grading system of tumor, TNM staging and bone metastasis)for predicting prognosis of prostate adenocarcinoma

MATERIALS AND METHODS:

- **Study design**: Observational cross-sectional study
- **Study setting**: The study was conducted in the Department of Pathology and Lab Medicine, AIIMS Bhopal, after approval from the ethics committee
- **Study period**: The Department of Pathology and Lab Medicine, AIIMS, Bhopal, received samples between January 2018 and June 2023. These samples were used in the study.
- **Sample size & sample collection**: A total sample size of 75 cases satisfying the inclusion criteria during the study period were included in the study. These cases include 60 core biopsies, 9 radical prostatectomy specimens & 6 TURP specimens.

INCLUSION CRITERIA :

1. All cases diagnosed with prostate adenocarcinoma on a histopathological basis by H and E slide examination during the study period in AIIMS Bhopal.
2. The cases diagnosed with prostate intraepithelial neoplasia on the basis of histopathology by H and E slide examination during the study period in AIIMS Bhopal

EXCLUSION CRITERIA:

1. All cases suspected of prostate cancer and microscopically determined to have Benign prostatic hyperplasia or prostatitis are excluded from the study.
2. Any cases with insufficient tissue materials in paraffin embedded tissue blocks are excluded from the study.
3. All cases with damaged paraffin embedded tissue blocks have been excluded from the study.
4. All cases in which consent cannot be acquired are excluded from the study.

Ethics approval: Ethics approval was obtained from the Institutional Human Ethics Committee for ethics approval. LOP No: IHEC-PGR/2021/PG/Jul/12 dated 28-02-2022

Statistical Analysis:

- Data was coded, and an MS Excel spreadsheet with the necessary parameters was created using the patient data that had been collected. The R language was used for the analysis, which was done in R studio.
- All statistical analysis will be performed using SPSS version 22 software (IBM Corp)
- Descriptive statistics were elaborated for continuous variables as means/standard deviations and medians/IQR, whereas for categorical variables, frequencies and percentages were used.
- When appropriate, data was displayed graphically using histograms, box-and-whisker plots, column charts, pie charts for continuous data and bar charts and pie charts for categorical data.
- The distribution of continuous variables was assessed using the Shapiro-Wilks test, and subsequent analyses were done according to the distribution.
- Pearson's Chi-square and Fisher's exact were used for the comparison of categorical variables.
- The chi-squared test will determine the association between Gleason's score and BRCA 1 and BRCA 2 expression.
- $P<0.05$ will be considered to indicate a statistically significant difference.

Methodology:

- Identifying the clinical, demographic, and histological details for each patient diagnosed with prostate cancer between January 2018 and June 2023.
- The study will include all prostate biopsies, TURP chips, and radical prostatectomy specimens that met the inclusion criteria and were received by the Department of Pathology and Lab Medicine at AIIMS Bhopal from the Department of Urology and the Department of Surgical Oncology between January 2018 and June 2023.
- All patients recruited in the study in prospective cases will be asked to sign a written informed consent form either during their hospital stay or at a subsequent visit. Waiver of consent is being asked for retrospective cases.
- All archived tissue blocks, slides, and patient reports related to prostate cancer will be retrieved. The pathologic requisition form containing clinical data (Patient's age, serum PSA) will be taken into consideration. It is essential to point out particular microscopic features, such as the Gleason score/New Contemporary Grading System of the tumour, lymphovascular invasion, perineural invasion, and metastasis.
- For retrospective and prospective patients, morphology-based histopathological diagnoses have been confirmed by slide analysis.
- All the specimens were fixed in 10% neutral buffered formalin.
- Gross examination findings were noted for all cases.
- Tissue was dehydrated in graded ethanol and paraffin-embedded
- Sections of 3 µm thickness were stained with hematoxylin and eosin.

Protocol for Hematoxylin and Eosin Staining

1) Xylene deparaffinization of the sections

2) Use graded alcohol in decreasing concentrations of 100%, 90%, and 75% to hydrate the tissue.

3) Hematoxylin staining for three to five minutes to identify the nucleus

4) Wash in running tap water for bluing

5) Differentiate in 1% acid alcohol

6) Wash in running tap water

7) Eosin Y counterstain for 10 minutes.

8) Rinse for one to five minutes under running tap water.

IHC was carried out on selected blocks of every case after interpreting histological features.

Figure 4.The automated immuno-stainer (VENTANA BenchMarkXT) used in the department of pathology and lab medicine, AIIMS Bhopal

On selected blocks of paraffin-embedded tissue, Automated IHC was carried out in accordance with the protocol listed below:

IHC with BRCA 1, BRCA 2 and Ki 67 antibodies on all the cases, a fully automated immunostainer (VENTANA BenchMarkXT) was used to perform IHC in a semi-automated manner by manually adding the antibodies. This machine can stain 30 slides in a single run and has independent slide processing drawers.

Positive control:

- For BRCA1, the human breast cancer tissue is considered as positive control where the nucleus of cells stains positive. The clone used was BRCA1 Purified mouse monoclonal antibody from Millipore (Mouse monoclonal clone MS13).
- For BRCA2, the tissue for positive control used was human breast and ovarian cancer. The cytoplasm and nucleus of the cells stain positive. The clone used was BRCA2 unconjugated mouse monoclonal antibody from Invitrogen (Mouse monoclonal clone MAF-234403).
- For ki67, tonsil tissue was used as positive control where the nucleus of cells of cells stain positive. The clone used was Ki 67 MK167/2462 mouse monoclonal antibody from Biogenex (Mouse monoclonal clone MK167/2462)

IHC was carried out using the Ultraview secondary antibody kit according to the protocol described below:

To start the machine, the computer is switched on double click on the Ventana icon and switch on the power button on the device.

1. Product registration: A registration probe connected to the computer was used to register products. This probe had to be touched twice to the metal memory button on the reagent pack to read and transfer the product information to the computer.
2. Reagent registration: The RFID button for the Ultraview DAB kit is located on the kit box. To register, select the Ventana software function button. Register Ventana Products by clicking. The RFIL button on the kit box, using the registration wand. For registration to be complete, click finalise.
3. Creating a protocol for a specific IHC marker: You can construct a template for a particular IHC marker by selecting the create new protocol option and entering the incubation period for that specific marker.
4. Generating barcode labels: Labels with barcodes were printed according to the antibody's predetermined protocol. To perform this, go to the "Home" page first, then press the "Create Label" button, the "Protocols button," the "Slide Labels" option, the "Label template," and protocols in that order.
5. Pre-Run Preparation: After product registration, as previously described, all reagent bottles were examined for reagent availability and, if empty, were refilled. The pH of the Reaction Buffer, EZ prep, and other buffers were checked, and the waste module was emptied if it was complete. Check the sensors are showing a green signal before the run.
6. Beginning a Run: Slide pads were washed by choosing the washing option. The reagent trays were loaded, and the reagent cap was removed, the reagents were set up in reagent slots on top of the staining machine in the same sequence as it was received. Then, load the hemotoxylin and bluing reagent.

7. Loading Slides: Before loading in the slide tray, the slides were put on the hot plate for 55 minutes to fix the tissue on the slides and deparaffinized them. Now, slides were kept in milk for 5 minutes to enhance blocking properties. The slide drawer is opened in the Instrument View, and slides are placed over the slide pads with the labels pointing up and inward. After ensuring that slides had to be appropriately positioned over the slide pad, close the tray. The number of drawer slides that have been installed is now counted and recorded in the system.
8. The running mode button was now pushed at the Instrument View. The system reads the bar codes on the reagent and slide, calculates the time until the primary antibody is applied, and begins the run.
9. The instrument will stop for adding primary antibodies, then open the slide tray and add 100 microlitres of assigned primary antibodies on a particular slide manually. Close the slide tray. Push the Ventana button located on the left side panel of the instrument.
10. Unload Completed Slides: When the run is completed, the drawer is opened, and stained slides are removed.
11. After Run: Now, slides were rinsed in warm, soapy water to remove any remaining reagents. Now, the slides were dried, and a coverslip was mounted using DPX and examined.

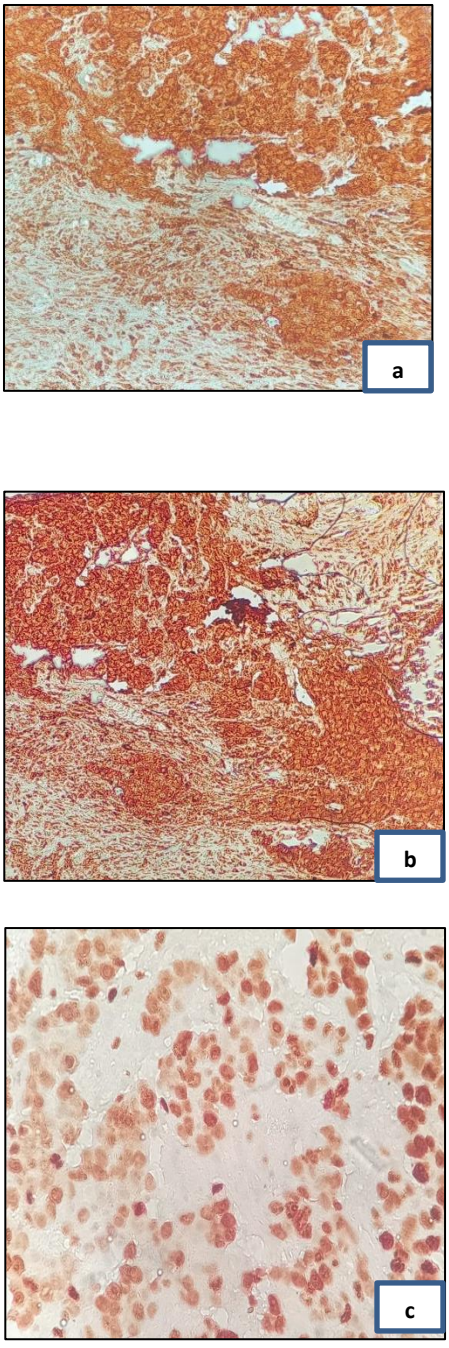


Figure. 5 a) Microphotograph showing breast cancer tissue , used as positive control for BRCA1 (IHC, 20X) b) Microphotograph showing breast cancer tissue , used as positive control for BRCA2 (IHC, 20X) c) Microphotograph showing tonsil tissue , used as positive control for Ki67 (IHC, 40X)

To identify the BRCA mutations, we employed the BRCA1 Purified mouse monoclonal antibody from Millipore (Mouse monoclonal clone MS13) and BRCA2 unconjugated mouse monoclonal antibody from Invitrogen (Mouse monoclonal clone MAF-234403).

Table 2: Table showing the antibody and dilution factor as per manufacturer's instructions.

Antibody	Clonality	Clone	Manufacturer	Quantity	Ready to use (RTU)/ Concentrated	Dilution
BRCA1	Mouse Monoclonal	MS13	Millipore	100 ug	Concentrated	1:100
BRCA2	Mouse Monoclonal	MAF-234403	Invitrogen	100 ug	Concentrated	1:100
Ki 67	Mouse Monoclonal	MK167/ 2462	Biogenex	3ml	Ready to use	

INTERPRETATION OF IHC :

When neoplastic nuclear staining was less than 10%, tumors were considered to have lost BRCA expression (BRCA IHC loss), and when staining was greater than 10%, they were considered to have retained BRCA expression (BRCA IHC intact). (96)

- ✓ In retained expression (Non carriers/Non mutants) - BRCA1 express nuclear staining, and BRCA2 expresses both cytoplasmic and nuclear staining of tumor cells.
- ✓ Loss of expression (Carriers/Mutants): When both the nucleus and cytoplasm of tumor cells are not stained, it is considered a loss of expression

	Retained expression (Non carriers/Non mutants)	**Loss of expression (Carriers/Mutants)**
BRCA 1		
BRCA 2		

Ki67:

With the help of 4x and 10x object lenses, the Ki-67 staining was evaluated to determine the "hot spot"—the location with the most intense staining. A 40x object lens was used to count at least 500 tumor cells in high-power fields to calculate the Ki-67 score. No matter how strongly stained a nucleus was, all the brown-stained nuclei were considered positive. According to the authors, a low proliferation index was defined as Ki-67 values less than 10% and a high proliferation index as Ki-67 values more than 10%. (97)

Ki67	**High proliferative index (>10%)**	**Low proliferative index (<10%)**

OBSERVATIONS & RESULTS :

An ambispective, observational study was performed in the Department of Pathology & Lab Medicine AIIMS on 75 specimens from patients who had visited the hospital complaining of lower urinary tract symptoms and histologically diagnosed as prostate carcinoma. The cases are considered following inclusion and exclusion criteria. Out of 75 specimens received, the majority of the specimens(80%) are core biopsies, followed by nine radical prostatectomy specimens. (12%) and 6 Transurethral resection of Prostate (TURP) specimens (8%)

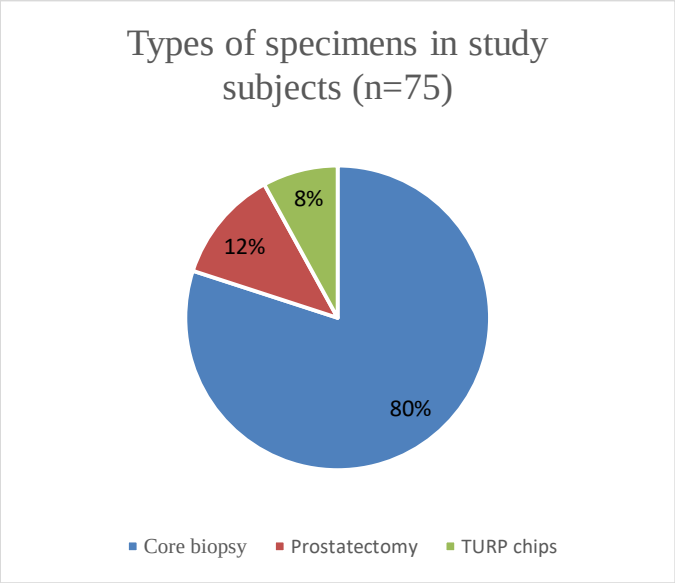


Figure 6 .Pie chart depicting the types of specimens in present study

Demographic profile of study participants:

Overall, patients with prostate cancer included in the current study ranged in age from 44 to 91 years, with the majority of cases occurring in the 61 to 70 year age group (32%) followed by 51 to 60 years (28%) and 71 to 80 years (26.7%). Out of the total 75 cases, the youngest age group patients of 41-50 years comprise four patients (5.3%), and the oldest age group of >80 years include six patients(8%). The mean age was calculated and found to be 65.87±10.55 years.

Age group	No. of cases	% of cases
41-50 years	4	5.3
51-60 years	21	28.0
61-70 years	24	32.0
71-80 years	20	26.7
>80 years	6	8.0

Mean age was 65.87±10.55 years

Table 3 . Age wise distribution of study participants (n=75)

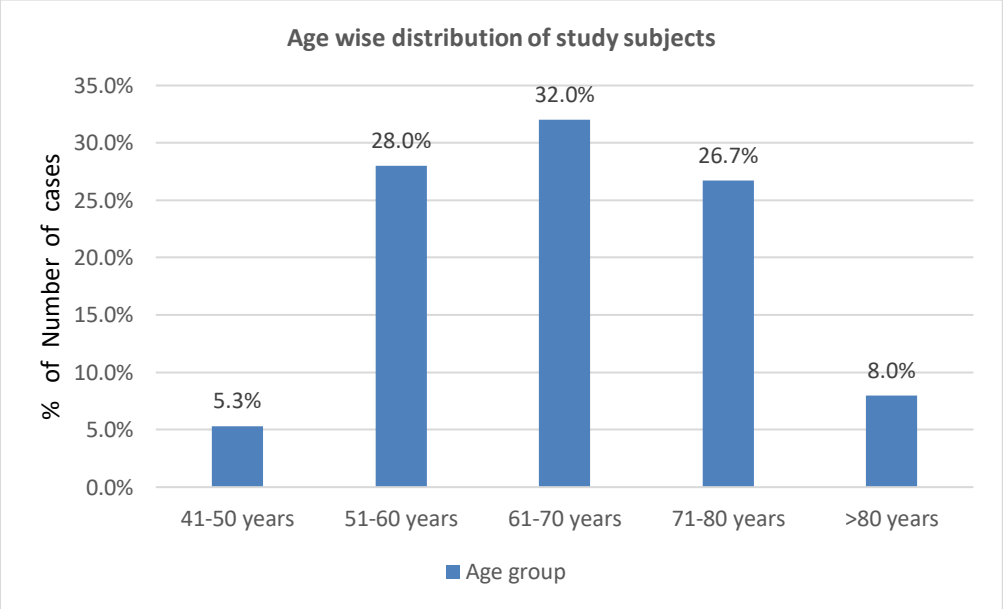


Figure 7 .Bar diagram displaying the age wise distribution of study subjects

Gleason score in study subjects:

A total of 75 cases were included in the study, with the majority having scores of 4+3=7 (20 cases—26.7%), 4+5=9 (20 cases—26.7%), and 4+4=8 (17 cases—22.7%). Eight cases scored 5+4=9, and five cases scored 3+4=7. Only one patient in the study obtained the maximum score of 5+5=10 (1.3%), and the lowest score was 3+3=6 in 4 cases (5.3%). Overall, 29 patients had a Gleason score <8 and 46 cases had a Gleason score ≥8.

Gleason score	**No. of cases**	**% of cases**
3+3=6	4	5.3
3+4=7	5	6.7
4+3=7	20	26.7
4+4=8	17	22.7
4+5=9	20	26.7
5+4=9	8	10.7
5+5=10	1	1.3

Table 4.Distribution of Gleason score in study subjects (n=75)

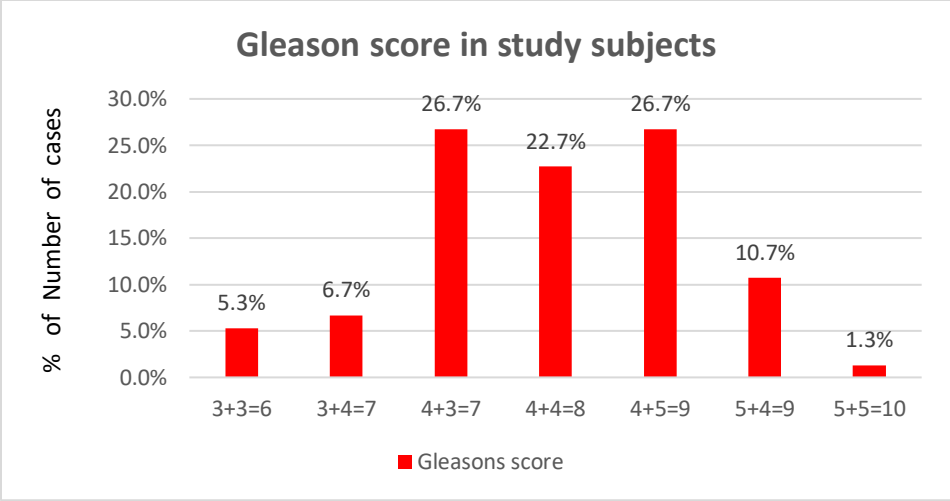


Figure 8.Bar diagram depicting the distribution of Gleason score in study subjects

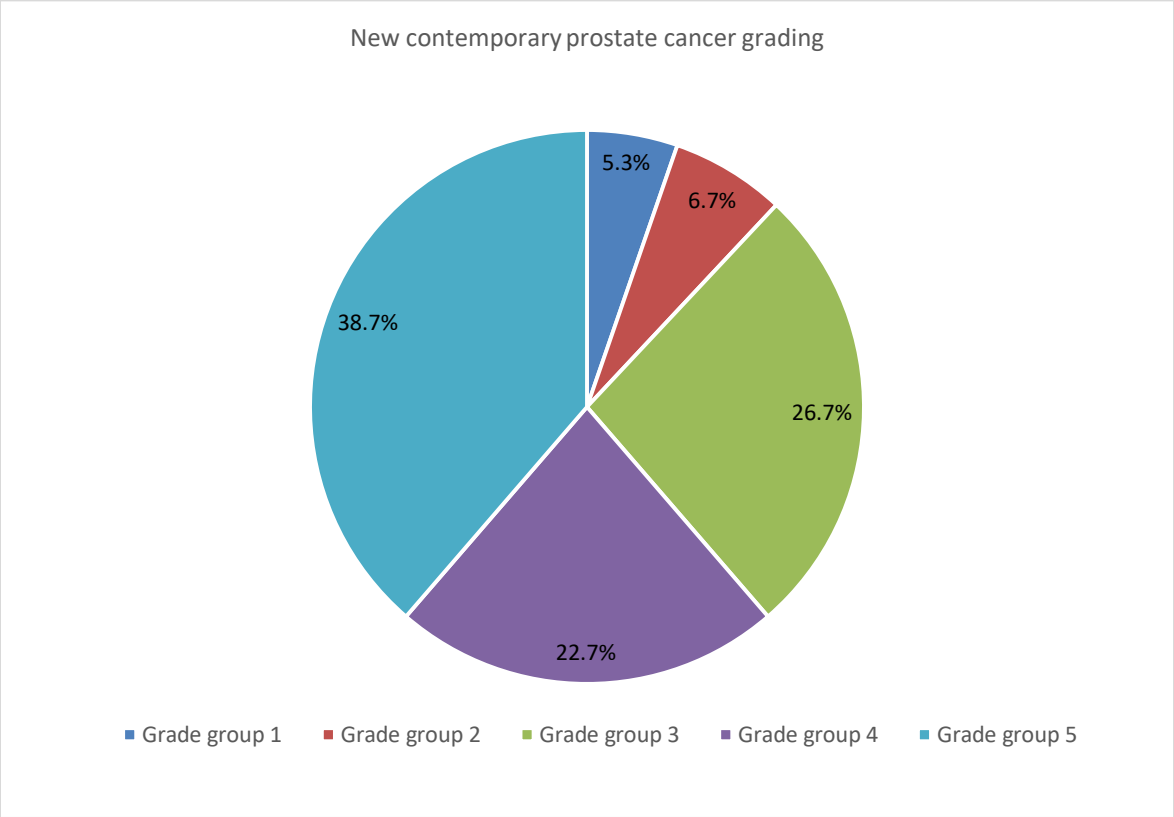
New contemporary prostate cancer grading in study subjects:

To avoid confusion & lack of proper detailing in the Gleason scoring, New contemporary prostate cancer grading was done for all 75 cases included in the study. A large proportion of study with 29 patients (38.7%) had the highest grade group 5. 20 cases and 17 cases had obtained grade groups of 3 & 4, respectively. Five cases got grade group 2 & four cases acquired grade group 1

New contemporary prostate cancer grading	No. of cases	% of cases
Grade group 1	4	5.3
Grade group 2	5	6.7
Grade group 3	20	26.7
Grade group 4	17	22.7
Grade group 5	29	38.7

Table 5. New contemporary prostate cancer grading in study subjects (n=75)

Figure 9. Pie chart displaying the distribution of New contemporary prostate cancer grading of cases



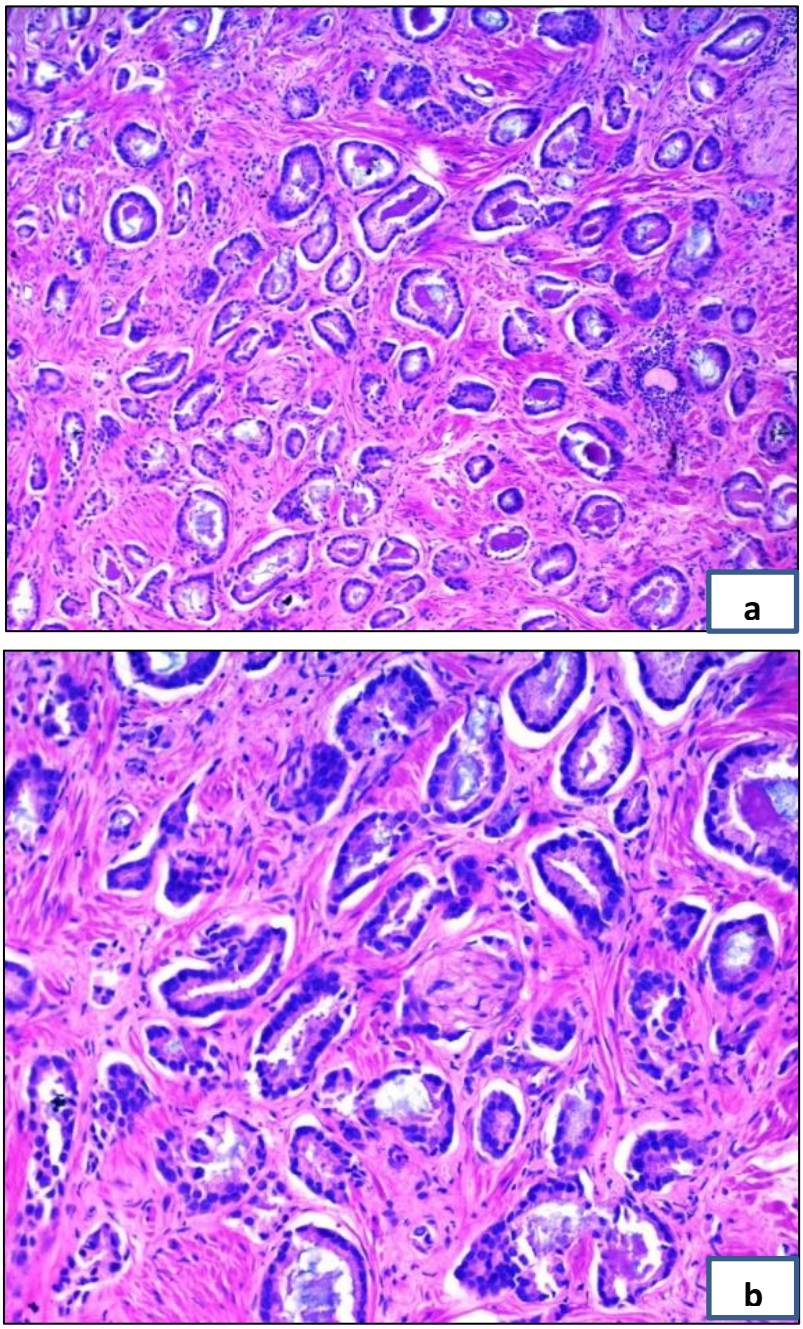


Figure. 10 a) Microphotograph showing numerous well formed individual glands and had a Gleason score 3+3=6 (H & E, 10X), b) Microphotograph of the same case with Gleason score 3+3=6 (H & E, 20X),

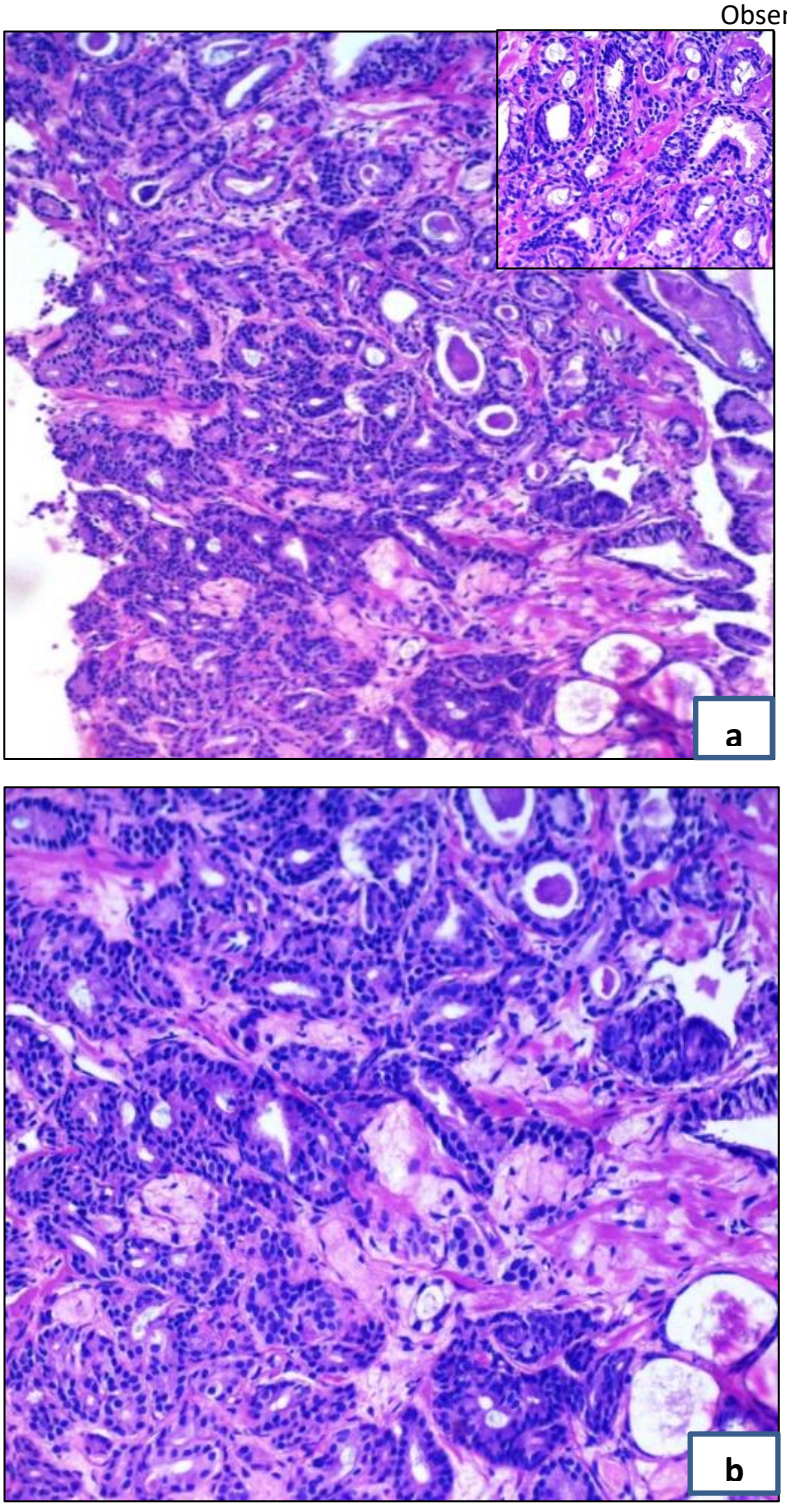


Figure. 11 a) Microphotograph showing case of Prostate Adenocarcinoma showing predominantly well formed individual glands with lesser component of poorly formed and fused glands (H & E, 10X) , Inset in right upper corner showing high magnification with both well formed and fused glands b) Microphotograph of the same case with similar features and designated a Gleason score 3+4=7 (H & E, 20X),

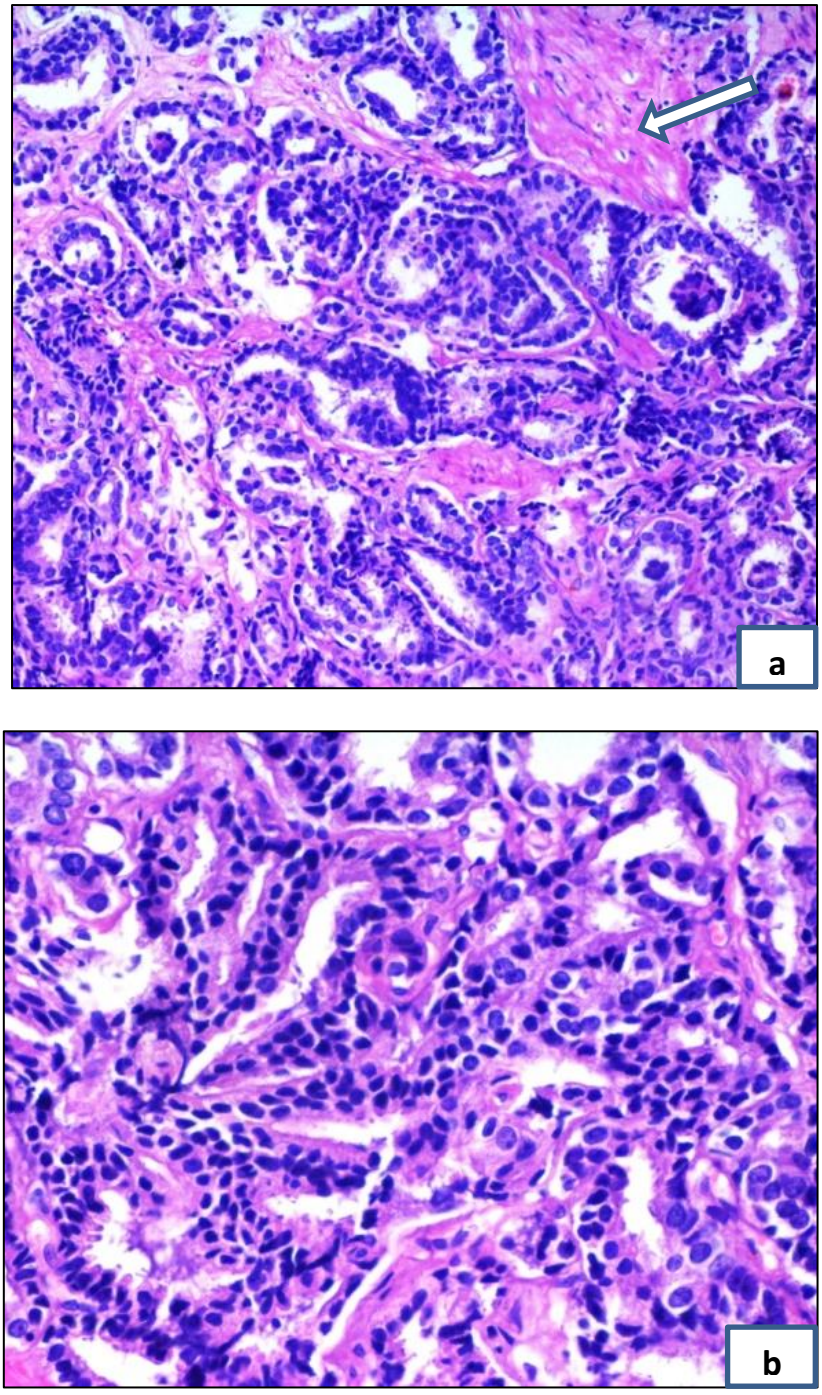


Figure. 12 a) Microphotograph showing core biopsy of Prostate Adenocarcinoma showing predominantly fused glands with lesser component of individual glands - Gleason score 4+3=7 (H & E, 20X) , Perineural invasion also identified (White arrow), b) Microphotograph of the same case with similar features and designated a Gleason score 4+3=7 – (H & E, 40X),

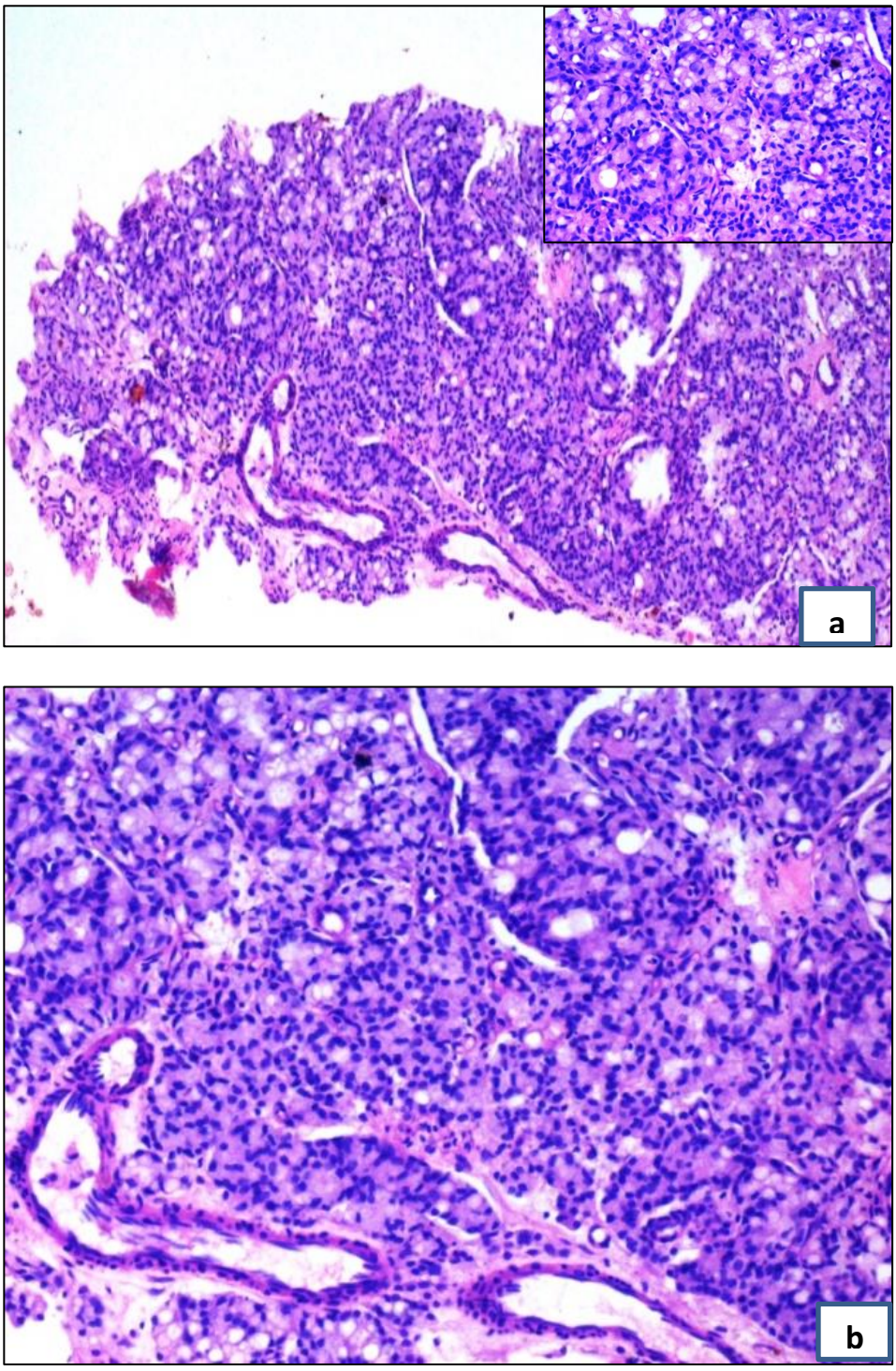


Figure. 13 a) Microphotograph showing with fused and poorly formed glands and had a Gleason score 4+4=8 (H & E, 20X) – Inset showing fused glands in higher magnification , b) Microphotograph showing the architecture of fused glands designated a Gleason score 4+4=8 (H & E, 40X)

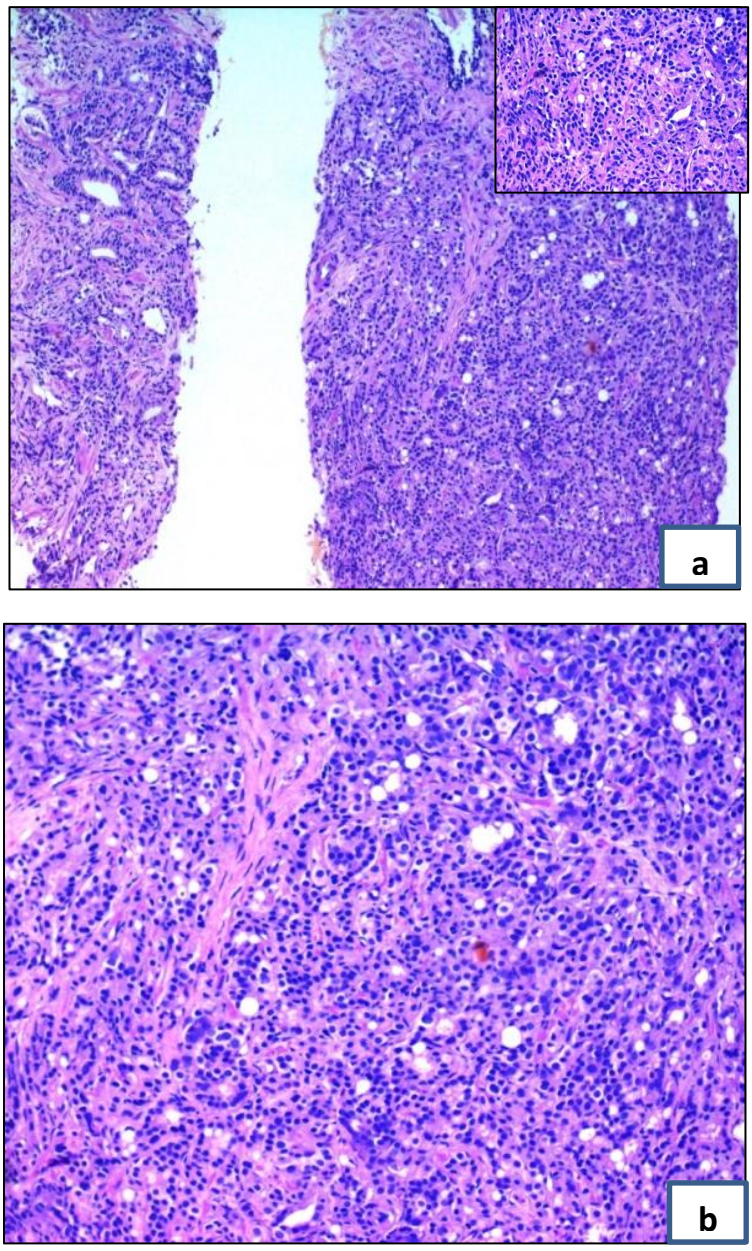


Figure. 14 a) Microphotograph showing core biopsy of prostate adenocarcinoma showing cords of tumor cells and few fused glands (H & E, 10X) , b) Microphotograph of the same case with tumor cells arranged in cords, singly scattered along with lesser component of fused glands , had Gleason score 5+4=9 (H & E, 20X)

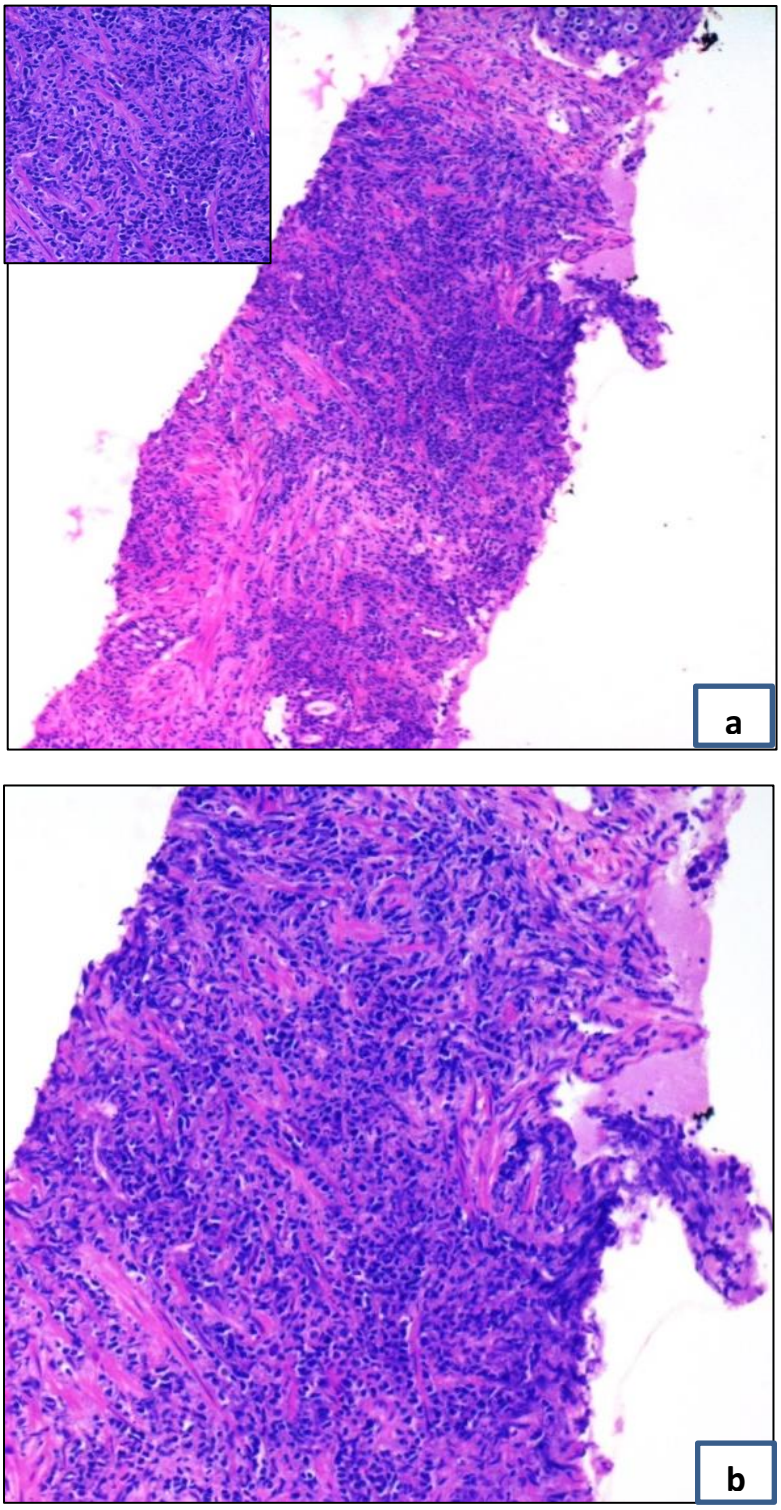


Figure. 15 a) Microphotograph showing core biopsy of prostate adenocarcinoma with cords of tumor cells in scanner view (H & E, 10X) , Inset showing cords and singly scattered tumor cells in higher magnification b) Microphotograph showing the same case with tumor cells arranged in cords and singly scattered designated a Gleason score 5+5=10 and Grade group 5 (H & E, 20X)

Lymphovascular invasion in study subjects:

Out of the 75 cases included in the present study, only 2.7% showed lymphovascular invasion, and the remaining 97.3% cases did not show any lymphovascular invasion.

Lympho-vascular invasion	No. of cases	% of cases
Not identified	73	97.3
Present	2	2.7

Table 6. Lympho-vascular invasion in study subjects (n=75)

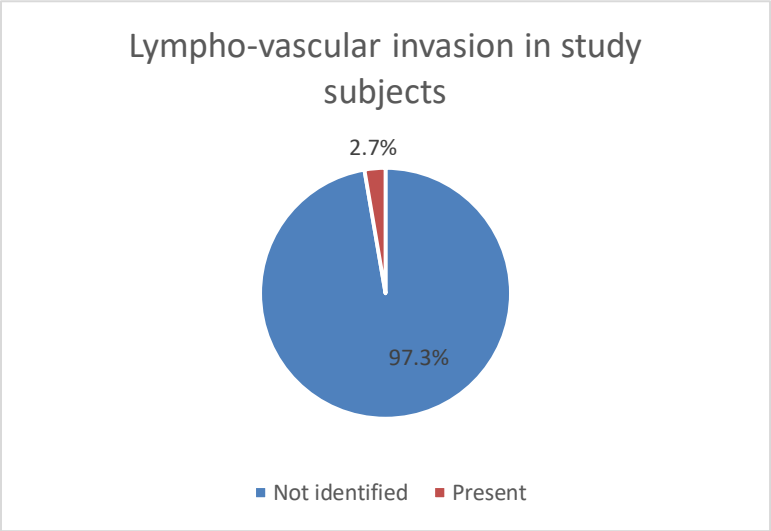


Figure 16.Pie chart depicting the lympho-vascular invasion in study subjects

Perineural invasion in study subjects:

According to Wu et al. Perineural invasion is defined as infiltration in any layer of the nerve sheath or tumor invasion involving at least one-third of the nerve circumference (98).

Perineural invasion is identified in more than half of the cases (46 cases- 61.3%) and not observed in the remaining 29 subjects (38.7%)

Perineural invasion	**No. of cases**	**% of cases**
Not identified	29	38.7
Present	46	61.3

Table 7. Peri neural invasion in study subjects (n=75)

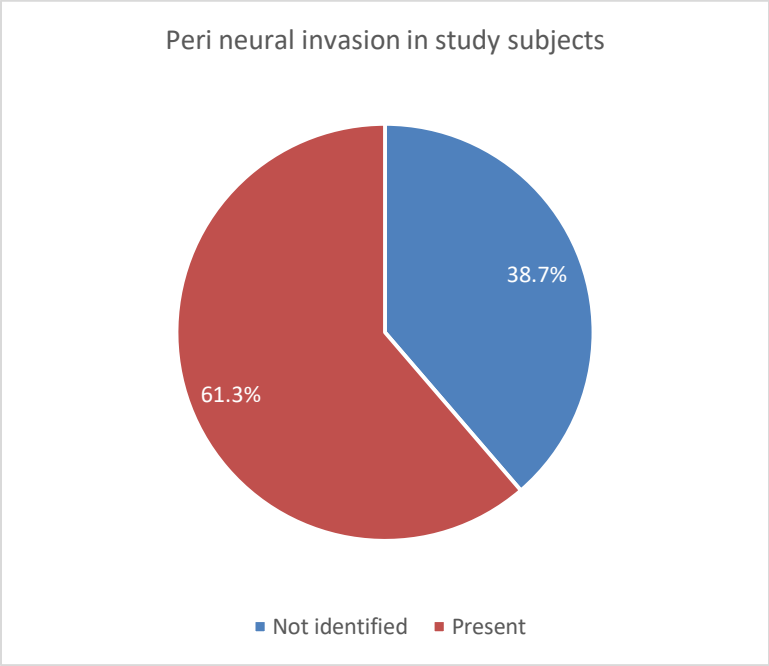


Figure 17. Pie chart depicting the Perineural invasion in study subjects

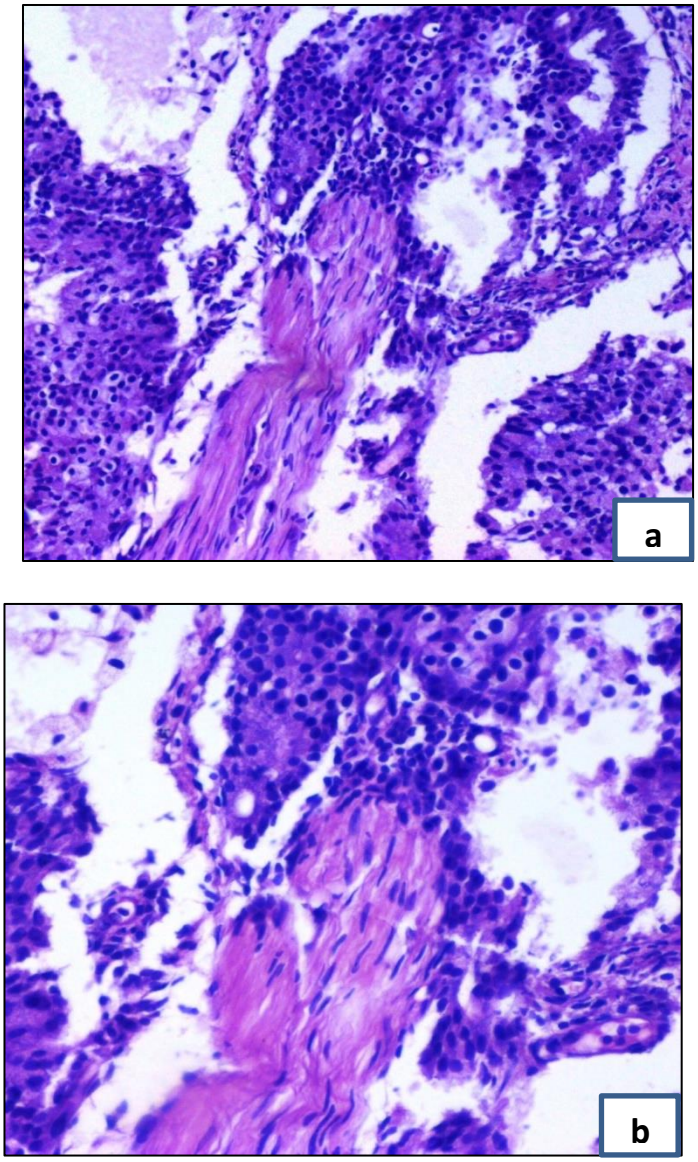


Figure. 18 a) Microphotograph showing perineural invasion of tumor cells (H & E, 20X), b) Microphotograph of the same case with perineural invasion using high power objective (H & E, 40X)

TNM staging in study subjects :

Out of the total 75 cases involved in the study, the T2 stage was observed in 41 cases, the T3 stage in 28 cases and the T4 stage in 6 cases. The T2 stage had subcategories of T2a,T2b and T2c and T3 has T3a and T3b.

TNM staging	No. of cases	% of cases
pT2a	11	14.7
pT2b	13	17.3
pT2c	17	22.7
pT3a	9	12.0
pT3b	19	25.3
pT4	6	8.0

Table 8. TNM staging in study participants (n=75)

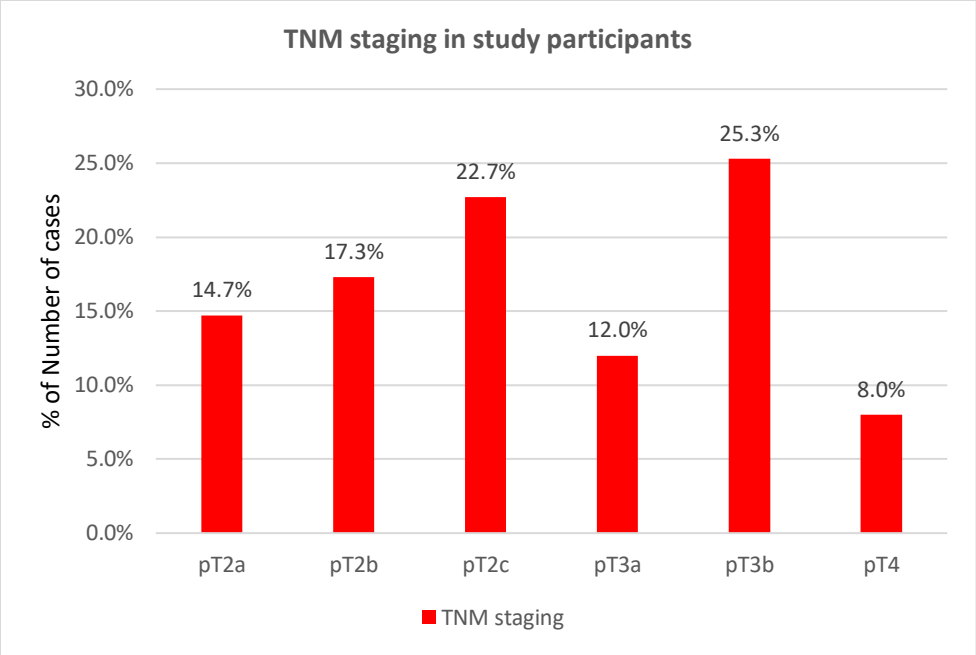


Figure 19.Bar diagram depicting the TNM staging in study participants (n=75)

Seminal vesicle involvement in study subjects

Out of the total 75 cases included in current study, 24 cases (32%) showed seminal vesicle involvement & 51 cases (68%) didn't showed seminal vesicle involvement

Seminal vesicle involvement	**No.**	**%**
Involved	24	32.0
Not involved	51	68.0

Table 9. Seminal vesicle involvement in study subjects (n=75)

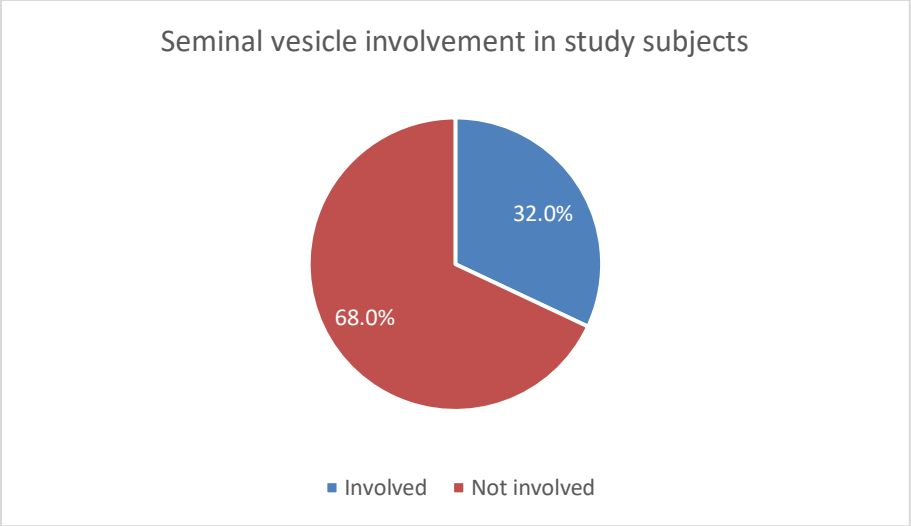


Figure 20. Pie chart depicting seminal vesicle involvement in study subjects

Regional lymph node involvement in study subjects

Out of the total 75 cases involved in the current study, eight patients (10.7%) showed regional lymph node involvement & 67 cases (89.3%) didn't show regional lymph node involvement. The regional lymph nodes include pelvic, external and internal iliac lymph nodes and obturator lymph nodes.

Regional lymph node involvement	**No.**	**%**
Involved	8	10.7
Not involved	67	89.3

Table 10. Regional lymph node involvement in study subjects (n=75)

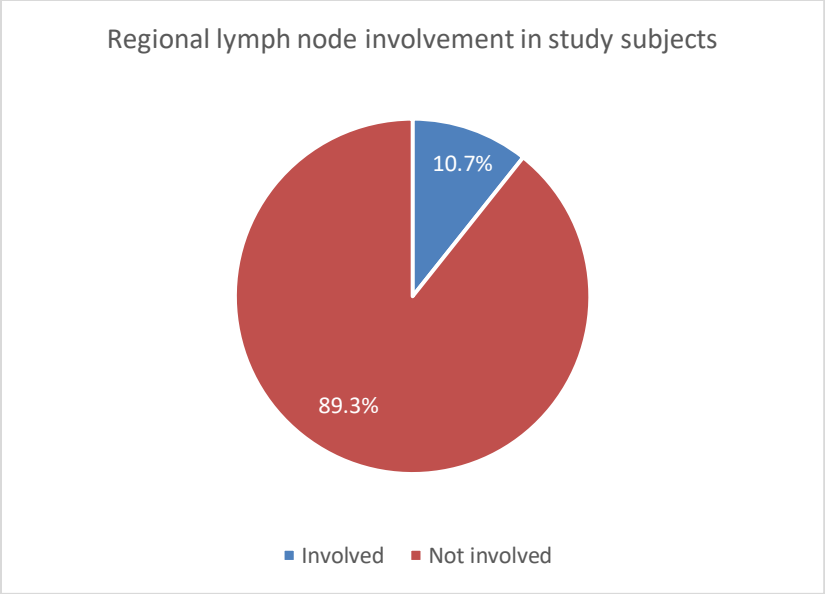


Figure 21.Pie chart displaying the regional lymphnode involvement in study subjects (n=75)

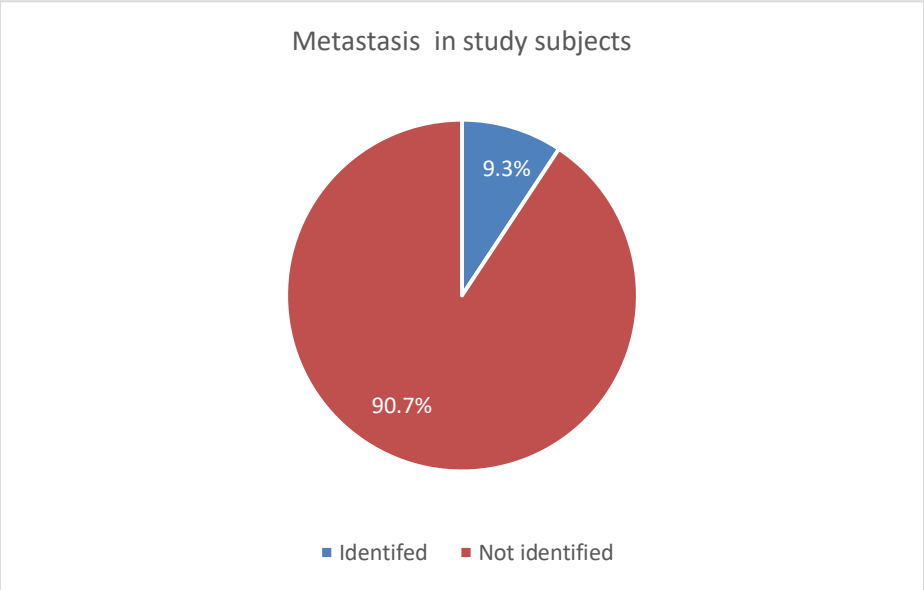
Metastasis in study subjects:

A total of 75 cases were involved in the present study. Out of them, 7 cases (9.3%) showed bony metastasic deposits of prostate carcinoma & 68 cases didn't show any metastasis. These data were obtained through radiological investigations during the follow-up visit of patients.

Table 11.Metastasis in study subjects (n=75)

Metastasis	**No.of cases**	**% of cases**
Identified	7	9.3
Not identified	68	90.7

Figure 22.Pie chart depicting presence/absence of metastasis in study subjects



Prevalence of BRCA1 expression in study subjects:

Of the total 75 cases in the study, every case underwent IHC analysis. Only 3 cases (4%) of the entire study population showed loss of expression of BRCA1, and the remaining 72 cases (96%) showed retained expression of BRCA1. The cases with loss of expression were considered as Mutants/carriers, and those with retained expression were considered as Non mutants/Non-carriers.

BRCA1 expression	**No.**	**%**
Loss of expression (Mutants/Carriers)	3	4.0
Retained expression (Non mutants /Non carriers)	72	96.0

Table 12.Table depicting prevalence of BRCA1 expression in study subjects (n=75)

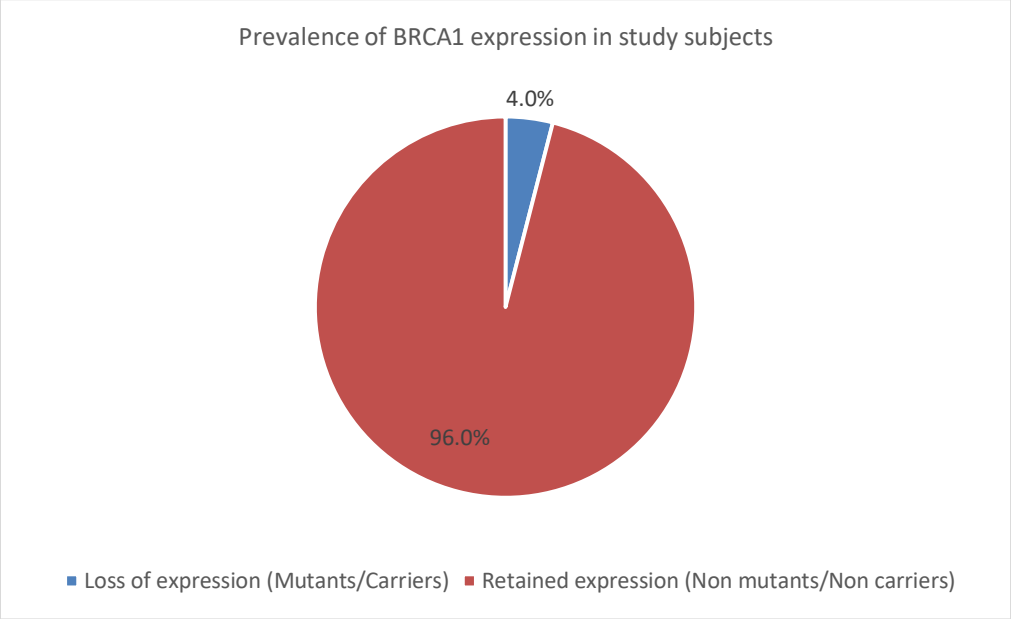
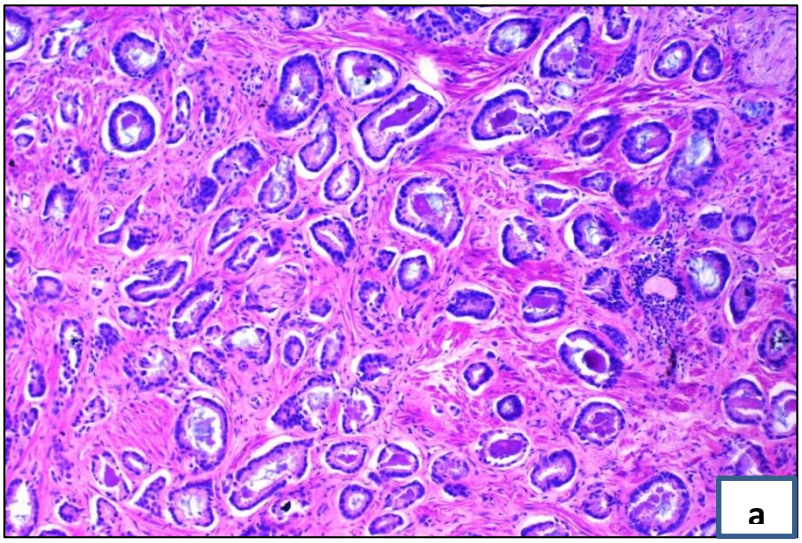


Figure 23.Pie chart depicting prevalence of BRCA1 expression in study subjects



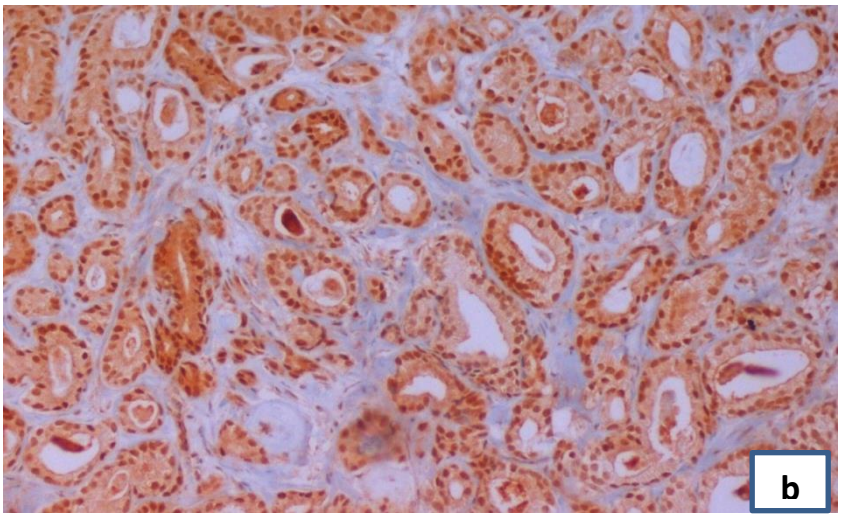


Figure 24.a) Microphotograph showing prostatectomy specimen of prostate adenocarcinoma showing numerous well formed glands with Gleason score of 3+3=6 (H & E, 40X) , b) Microphotograph of the same case with similar features and showing retained BRCA1 expression (nuclear staining) (IHC, 40X)

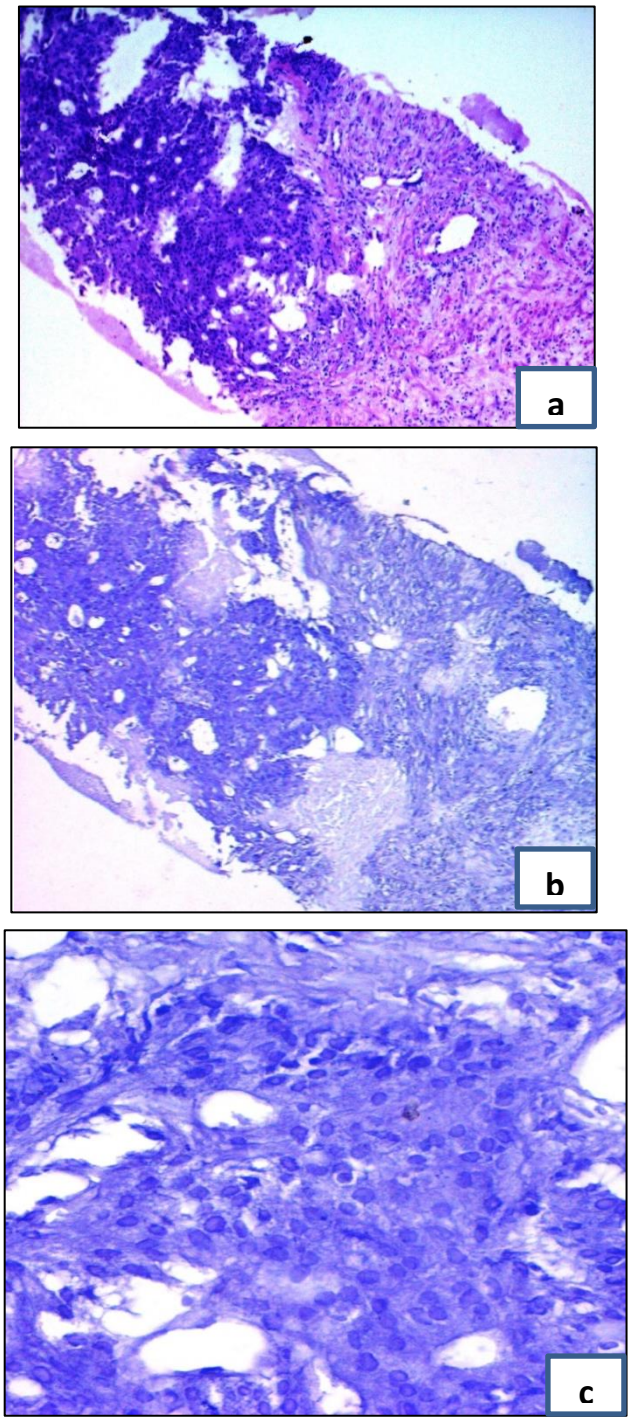


Figure 25. a) Microphotograph showing core biopsy of prostate adenocarcinoma showing components of fused and poorly formed glands along with lesser component of singly scattered tumor cells, assigned a Gleason score of 4+5=9 and grade group 5 (H & E, 10X) , b) Microphotograph showing the same case with tumor cells showing loss of expression of BRCA1 (IHC,10X), c) Microphotograph of the same case with tumor cells showing loss of expression of BRCA1 (complete absence of staining) (IHC, 40X)

Prevalence of BRCA2 expression in study subjects:

Out of the 75 cases, only 4 (5.3%) exhibited loss of BRCA2 expression, while the remaining 71 (94.6%) showed retained expression of BRCA2. The cases with loss of expression were considered as Mutants/carriers, and those with retained expression were considered Non mutants /Non-carriers.

BRCA2 expression	**No.**	**%**
Loss of expression - Mutants/ Carriers	4	5.3
Retained expression - Non mutants / Non carriers	71	94.7

Table 13. Table depicting prevalence of BRCA2 expression in study subjects (n=75)

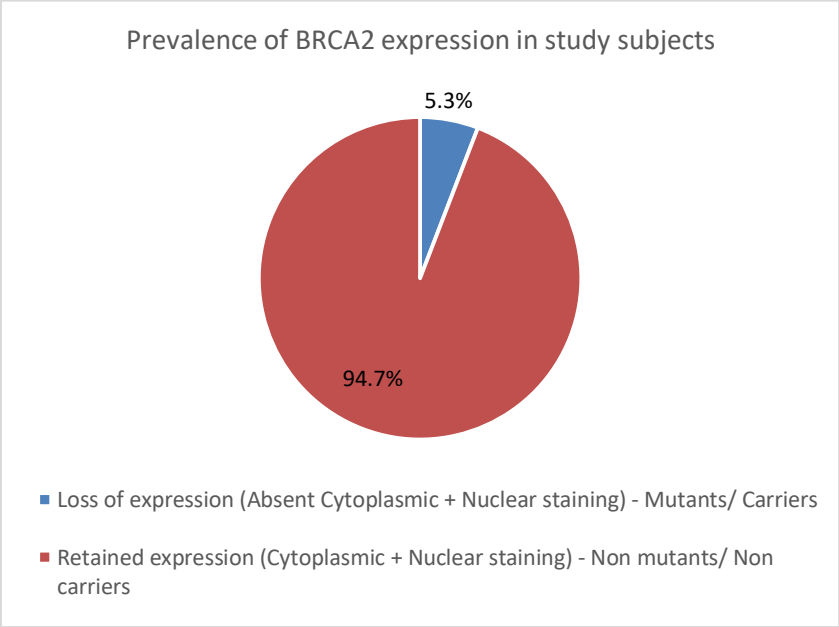

Prevalence of BRCA2 expression in study subjects
5.3%
94.7%
Loss of expression (Absent Cytoplasmic + Nuclear staining) - Mutants/ Carriers
Retained expression (Cytoplasmic + Nuclear staining) - Non mutants/ Non carriers

Figure 26.Pie chart depicting prevalence of BRCA2 expression in study subjects

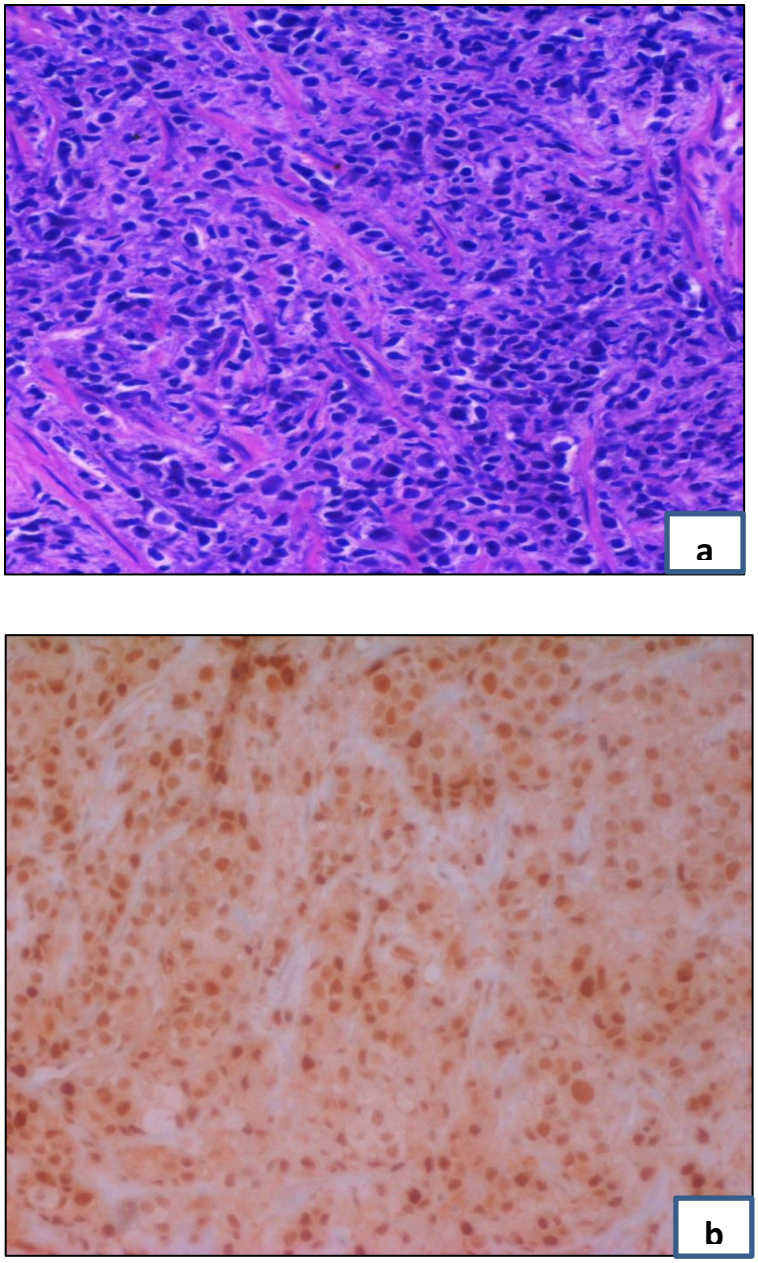


Figure 27. a) Microphotograph of core biopsy of prostate adenocarcinoma with both sheets of tumor cells and singly scattered tumor cells (H & E, 40X), b) Microphotograph showing the same case with retained BRCA2 expresssion (nuclear and cytoplasmic staining) (IHC, 40X)

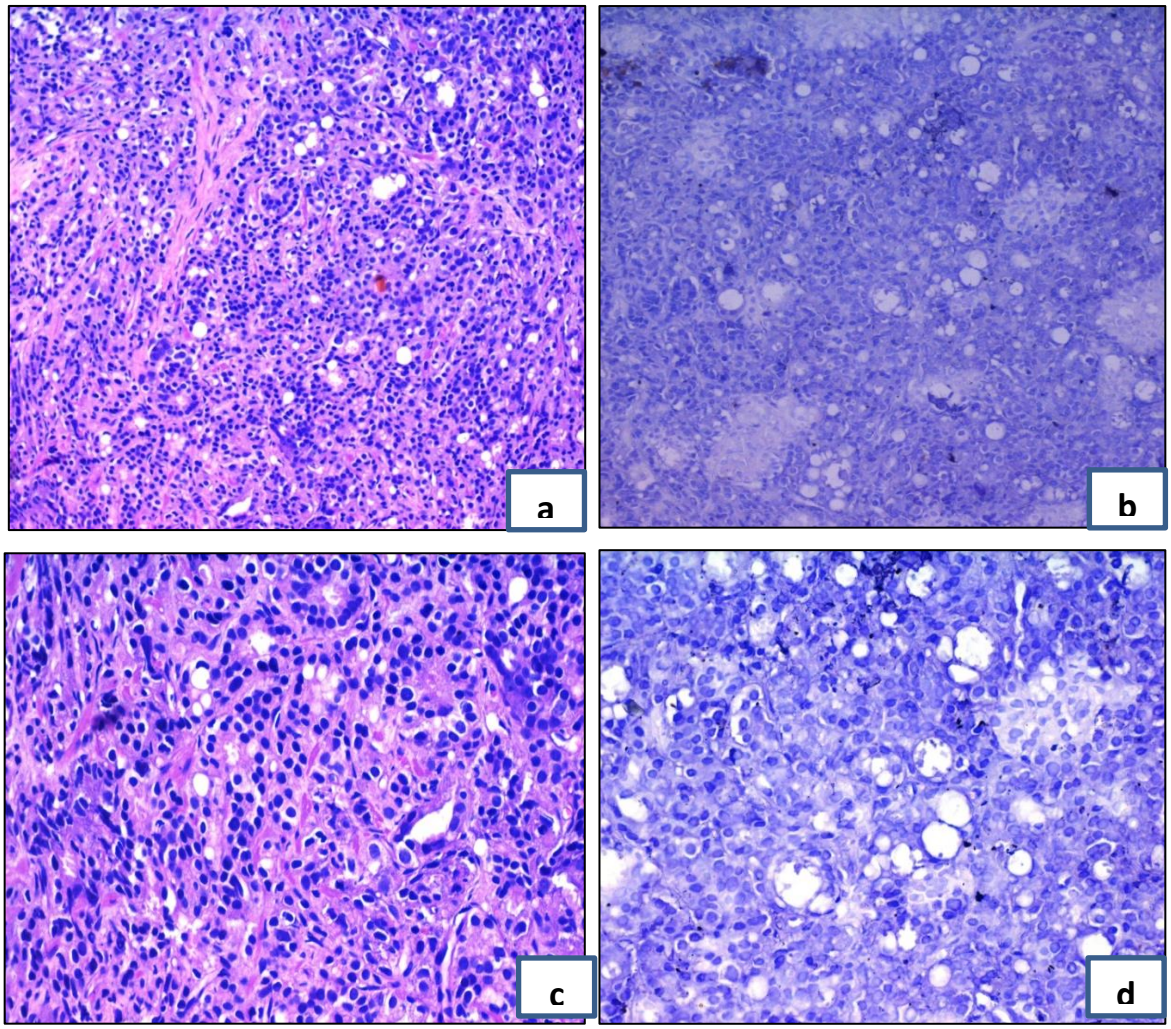


Figure 28.a) Microphotograph of the Prostate adenocarcinoma case with tumor cells arranged in sheets, singly scattered along with lesser component of fused glands , had Gleason score 5+4=9 (H & E, 20X), b) Microphotograph showing the same case with loss of expression of BRCA2 (complete absence of staining) (IHC, 20X) c) Microphotograph of the same case with tumor cells arranged in sheets, singly scattered along with lesser component of fused glands , had Gleason score 5+4=9 (H & E, 40X), d) Microphotograph showing the same case with loss of expression of BRCA2 (complete absence of staining) (IHC, 40X)

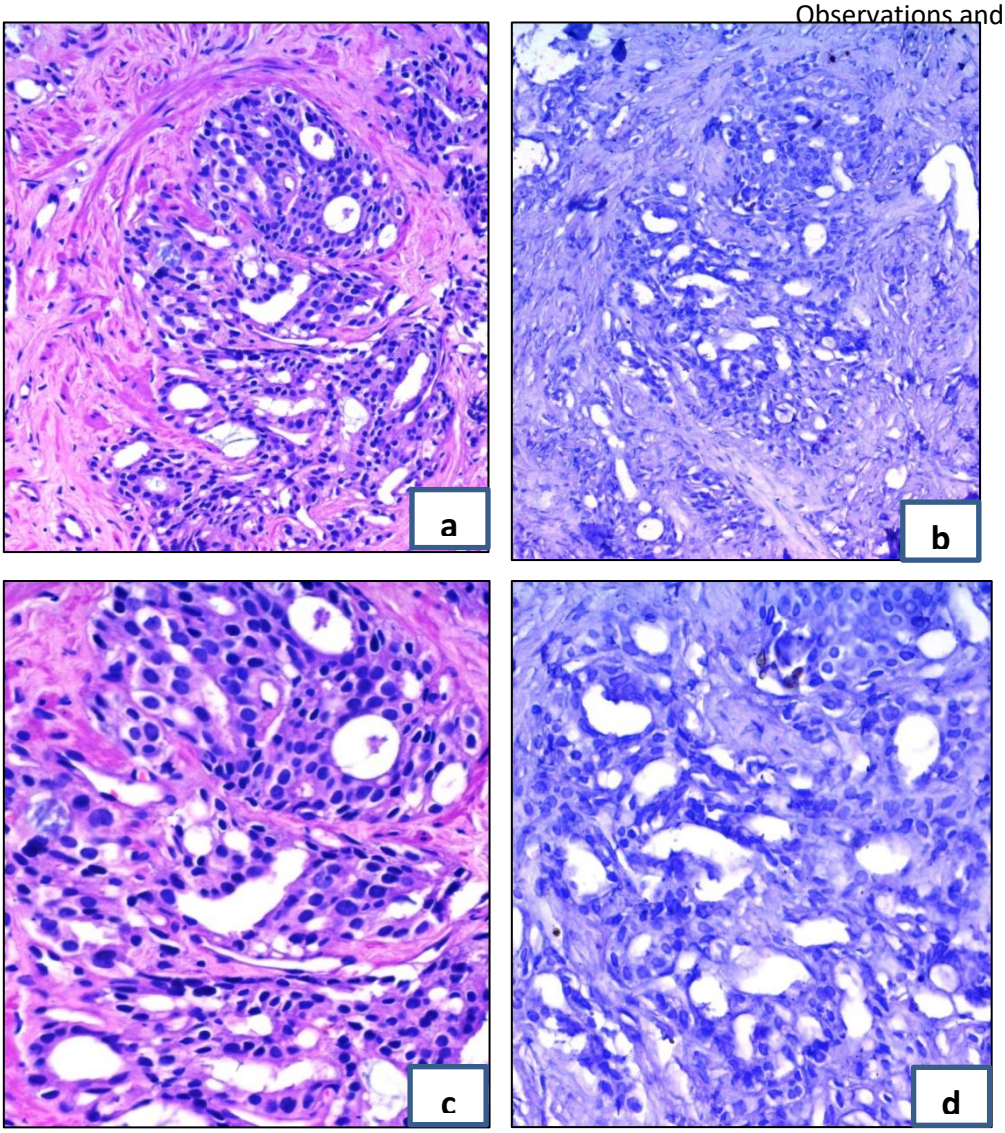


Figure 29. a) Microphotograph showing radical prostatectomy specimen diagnosed as Prostate Adenocarcinoma showing cribriform pattern of tumor cells, with Gleason score of 4+4=8 , Grade group 4 (H & E, 20X) , b) Microphotograph of the same case with tumor cells in cribriform arrangement and loss of BRCA2 expression (IHC, 20X), c) Microphotograph of the same case with cribriform pattern of tumor cells in high power objective (H & E, 40X), d) Microphotograph of the same case with loss of expression of BRCA2 (complete absence of staining) (IHC, 40X)

Ki67 status of study subjects

Out of the total 75 cases involved in the study, 17 cases (22.7%) had been identified as cases with high proliferative index & 58 cases were identified as cases with low proliferative index.

Table 14. Table depicting the Ki67 status of study subjects (n=75)

Ki67	No.	%
High proliferative index	17	22.7
Low proliferative index	58	77.3

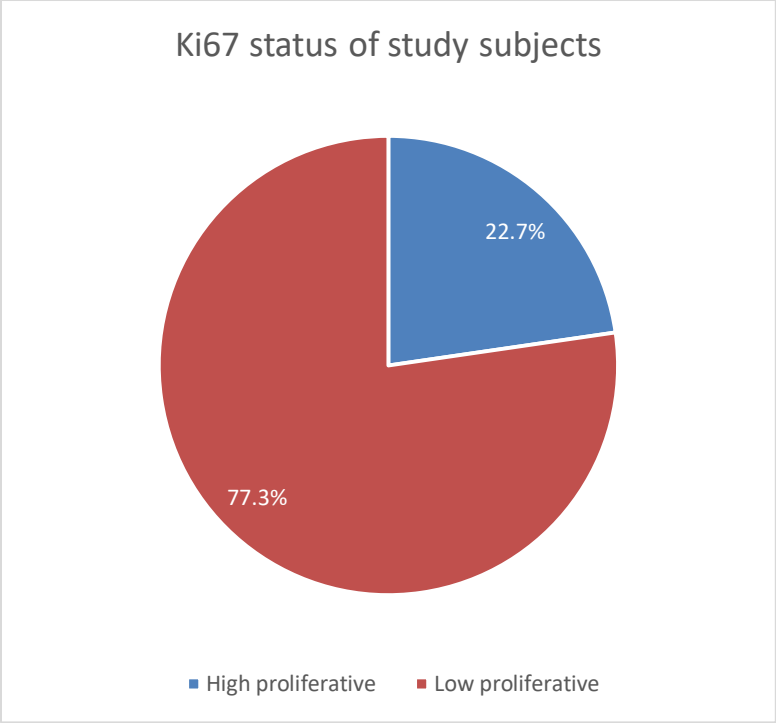


Figure 30.Pie chart showing activity of Ki67 status in study subjects

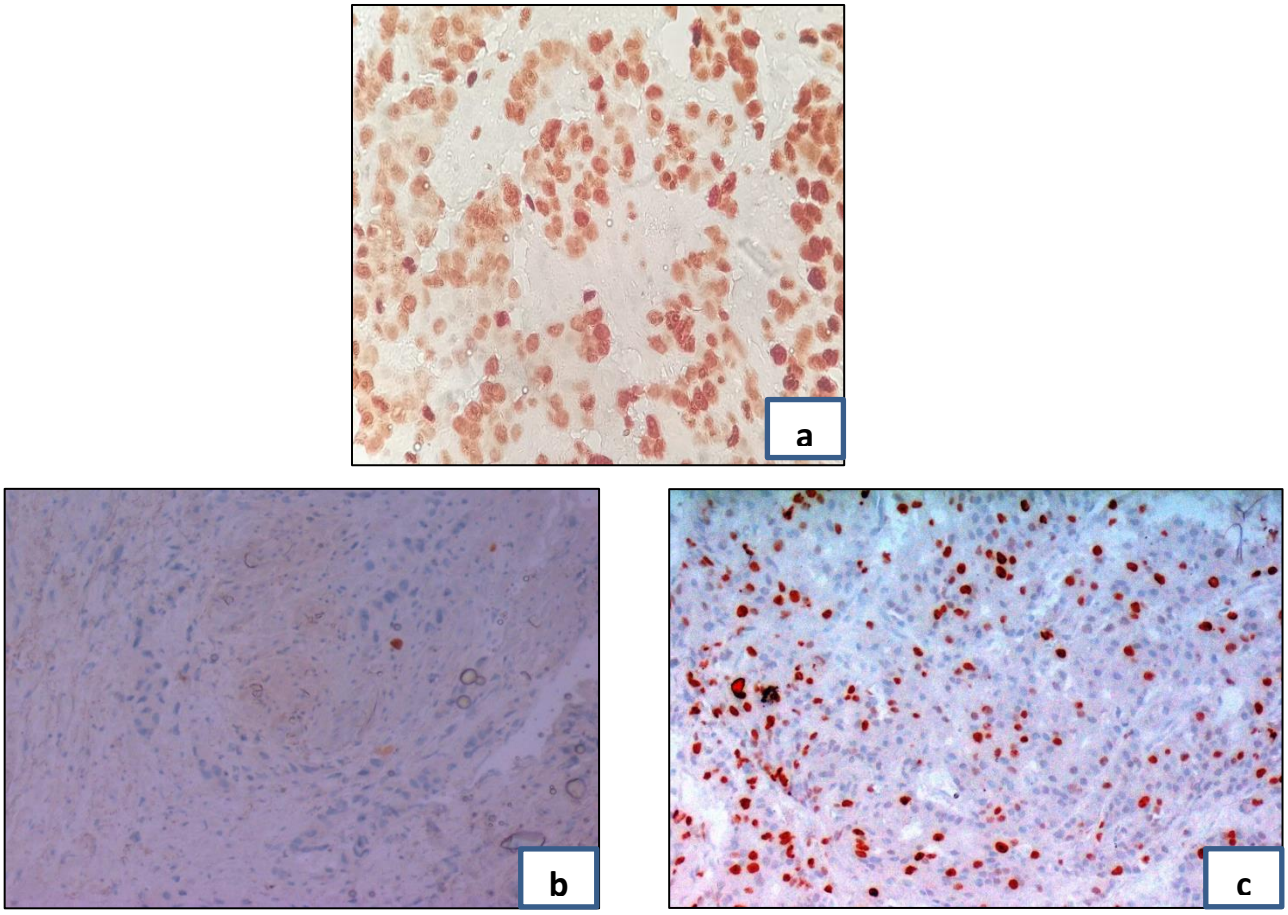


Figure. 31 a) Microphotograph showing tonsil tissue , used as positive control or Ki67 (IHC, 40X),b)Microphotograph showing case of prostate adenocarcinoma showing low Ki67 proliferative index <10% (IHC, 20X) , c) Microphotograph of case of prostate adenocarcinoma with high Ki67 proliferative index ≥10% (IHC, 20X)

CORRELATIONS & ASSOCIATIONS

Correlation of BRCA Expression with various clinicopathological Variables:

Association between Age & BRCA1 expression:

In the present study, 75 patients diagnosed with prostate adenocarcinoma underwent IHC analysis. The age of patients is correlated with BRCA1 expression using the Chi-square test.

It was observed that 66.7% of cases with lost BRCA1 expression (carriers) and only 32% of cases with retained BRCA expression presented at younger age (<60 years). These results suggested that the patients with loss of BRCA1 expression (Mutants/carriers) present at younger age than Nonmutants/non carriers. However, no statistically significant difference was found between age and BRCA1 expression (p=0.57 for BRCA1)

Age group	**Loss of BRCA1 expression**	**Retained BRCA1 expression**	**p value**
41-50 years	0	4 (5.6%)	0.57
51-60 years	2 (66.7%)	19 (26.4%)	
61-70 years	1 (33.3%)	23 (31.9%)	
71-80 years	0	20 (27.8%)	
>80 years	0	6 (8.3%)	

Chi square test used

Table 15. Table depicting association between age and BRCA1 expression

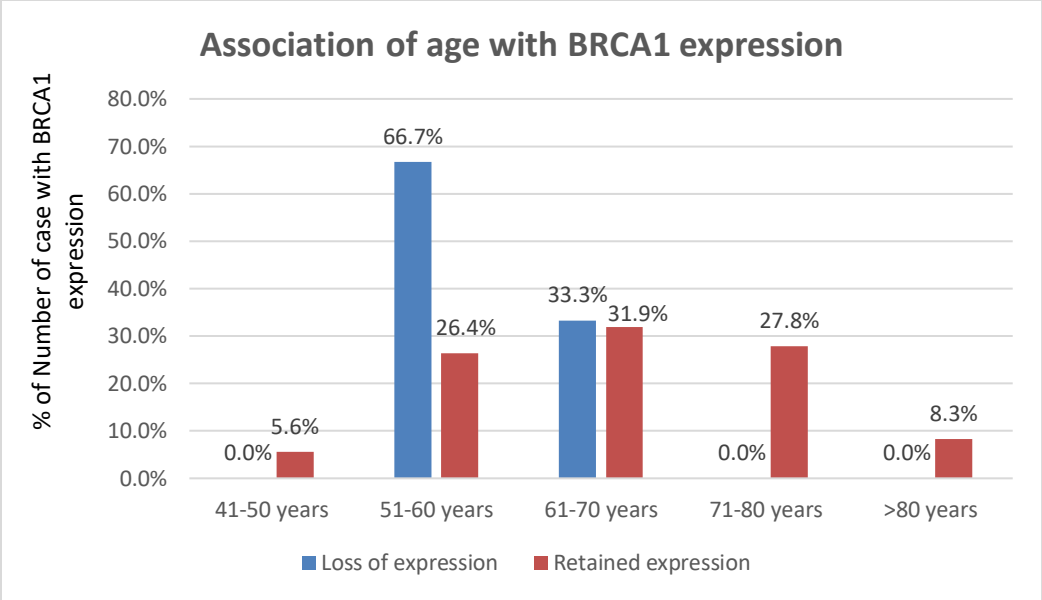


Figure 32.Component bar graph depicting association of age with BRCA1 expression

ASSOCIATION BETWEEN AGE & BRCA2 EXPRESSION:

Using the Chi-square test, the age of the patients is correlated with the expression of BRCA2. It is found that 66.7% of cases with lost BRCA2 expression presented at a young age, while in cases with retained BRCA2 expression, only 32% presented at a younger age. These findings imply that patients who have lost BRCA2 expression (mutants or carriers) manifest at a younger age than non-mutants or non-carriers. No statistically significant difference was found between age and BRCA2 expression (p=0.27 for BRCA2).

Age group	Loss of BRCA2 expression	Retained BRCA2 expression	p value
41-50 years	0	4 (5.6%)	
51-60 years	3 (66.7%)	18 (25.4%)	0.27
61-70 years	1 (33.3%)	23 (32.4%)	
71-80 years	0	20 (28.2%)	
>80 years	0	6 (8.5%)	

Chi square test used

Table 16.Table depicting the association between age and BRCA2 expression

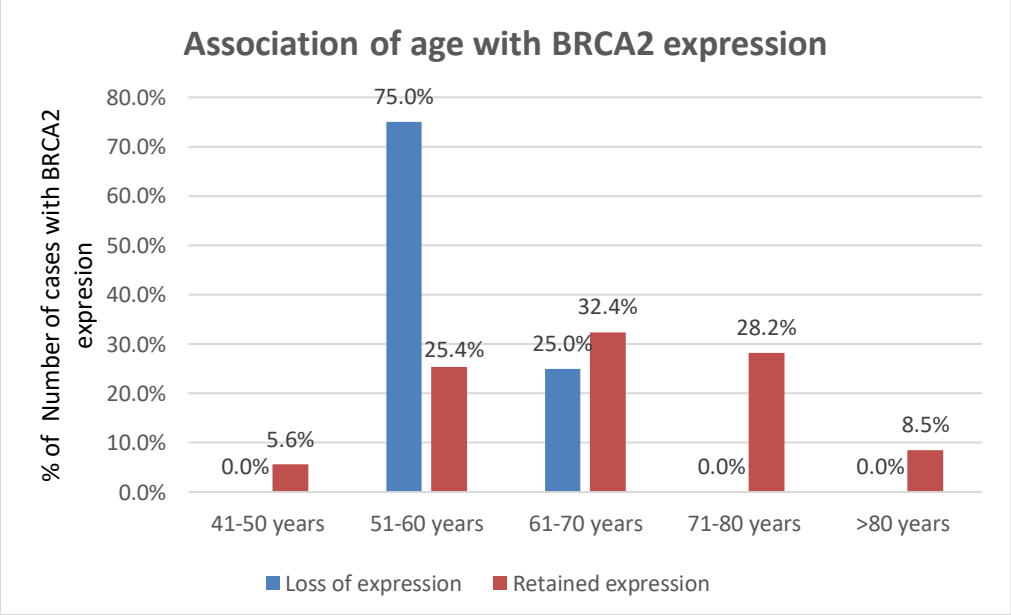


Figure 33.Component bar graph showing association of age with BRCA2 expression

ASSOCIATION BETWEEN SERUM PSA & BRCA1 EXPRESSION:

The mean PSA was calculated and found to be 89.03±26.69 for cases with loss of BRCA2 expression. Using Mann Whitney U test, the association between serum PSA and BRCA2 expression were correlated and found that the mean PSA of cases with loss of BRCA1 expression was more than that of subjects with retained expression but did not result in a statistically significant difference (p=0.31)

BRCA1 expression	**Mean serum PSA(ng/ml)**	**SD**	**p value**
Loss of expression	89.03	26.69	0.31
Retained expression	71.47	34.56	

Mann Whitney U test used

Table 17.Association between serum PSA and BRCA1 expression

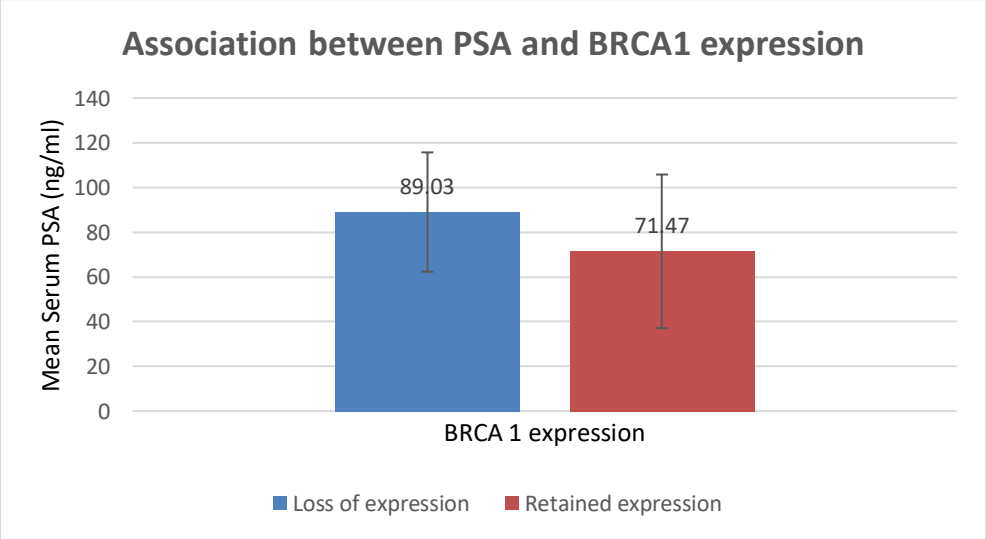


Figure 34.Association between mean PSA and BRCA1 expression

ASSOCIATION BETWEEN SERUM PSA & BRCA2 EXPRESSION:

Mean Prostate specific antigen was 70.39±34.35 in subjects with retained expression of BRCA2, and it was 103.82±10.09 in subjects with loss of expression. This difference was found statistically significant (p-value = 0.02), implying there is an association between PSA and BRCA2 expression. The mean PSA of the cases with loss of BRCA2 expression was higher than cases with retained BRCA2 expression.

Table 18.Table depicting association between Serum PSA and BRCA2 expression

BRCA2 expression	**Mean Serum PSA(ng/ml)**	**SD**	**p value**
Loss of expression	103.82	10.09	**0.02**
Retained expression	70.39	34.35	

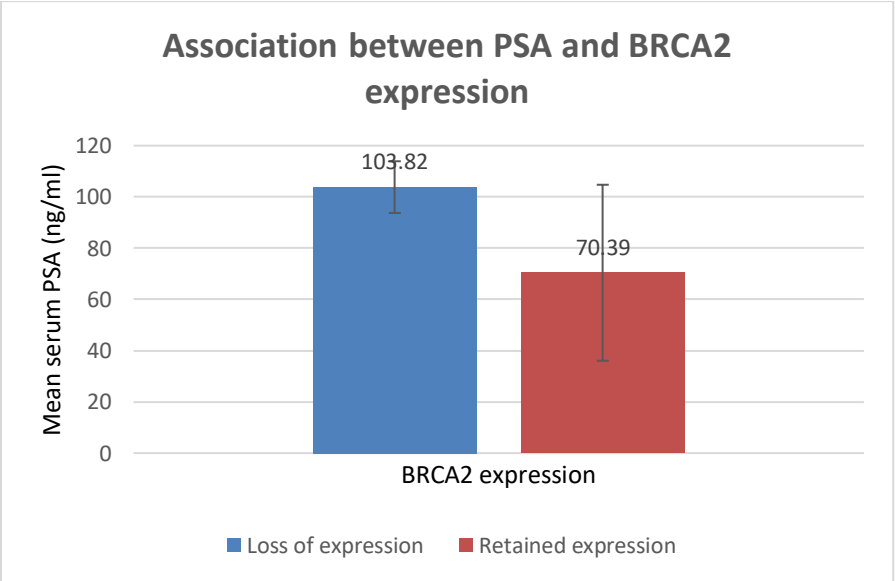


Figure 35. Association between PSA and BRCA2 expression

ASSOCIATION BETWEEN GLEASON SCORE & BRCA1 EXPRESSION:

Thompson et al.:- The cutoff Gleason scores of the prostate tumours were categorized into <8 and ≥8 subgroups. This cutoff was chosen based on clinical experience and prior literature suggesting that clinical outcome for Gleason score 8 prostate cancer is more similar to Gleason score 8 to 10 than for Gleason score <8

The association of the Gleason score with BRCA1 expression was determined using the Fisher exact test. Of all the 3 cases with loss of BRCA1 expression, 2 cases (66.7%) show Gleason score ≥8, 1 case (33.3%) had a Gleason score of less than 8, suggesting that cases with lost BRCA1 expression (carriers) had higher Gleason scores. However, the result was not found to have a statistically significant difference (p-value 1.0)

Table 19.Table depicting the association of Gleason score with BRCA1 expression

Gleason score	**Loss of expression**	**Retained expression**	**p value**
<8	1 (33.3%)	28 (38.9%)	1.0
≥8	2 (66.7%)	44 (61.1%)	

Fisher exact test used

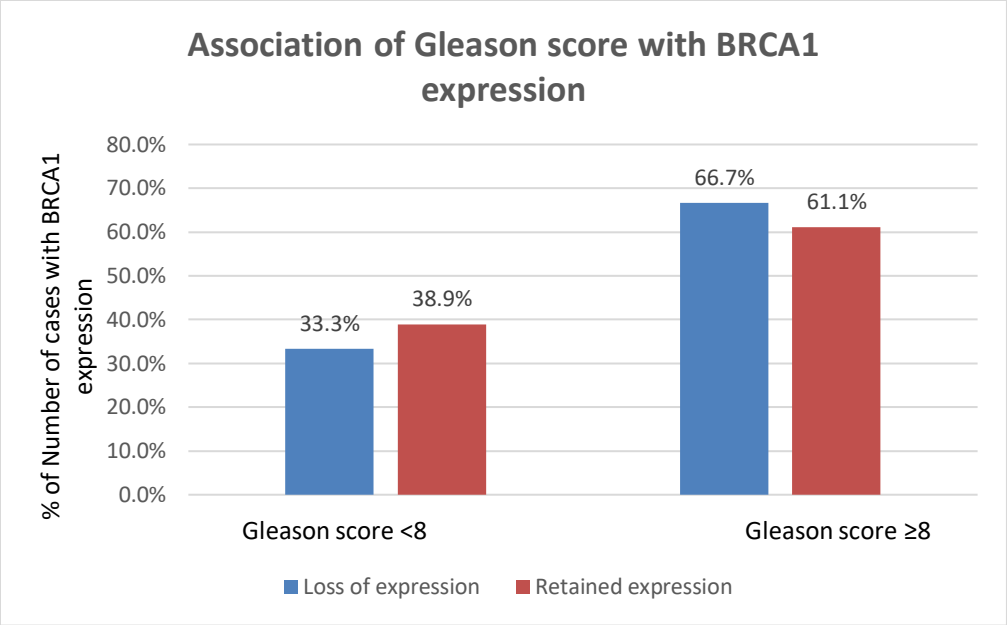


Figure 36.Component bar graph depicting association of Gleason score with BRCA1 expression.

ASSOCIATION BETWEEN GLEASON SCORE & BRCA2 EXPRESSION:

The Fisher exact test is used to study the relationship between Gleason score and BRCA2 expression. All the four cases (100%) with loss of BRCA2 expression had Gleason score ≥8. In contrast, only 59.2% of patients with retained expression showed a Gleason score ≥8, indicating that BRCA2 carriers had higher Gleason scores than non carriers. However, it turned out that there was no statistically significant difference in the result (p-value 0.15).

Table 20. Table depicting the association of Gleason score with BRCA2 expression

Gleason score	Loss of expression	Retained expression	p value
<8	0	29 (40.8%)	0.15
≥8	4 (100%)	42 (59.2%)	

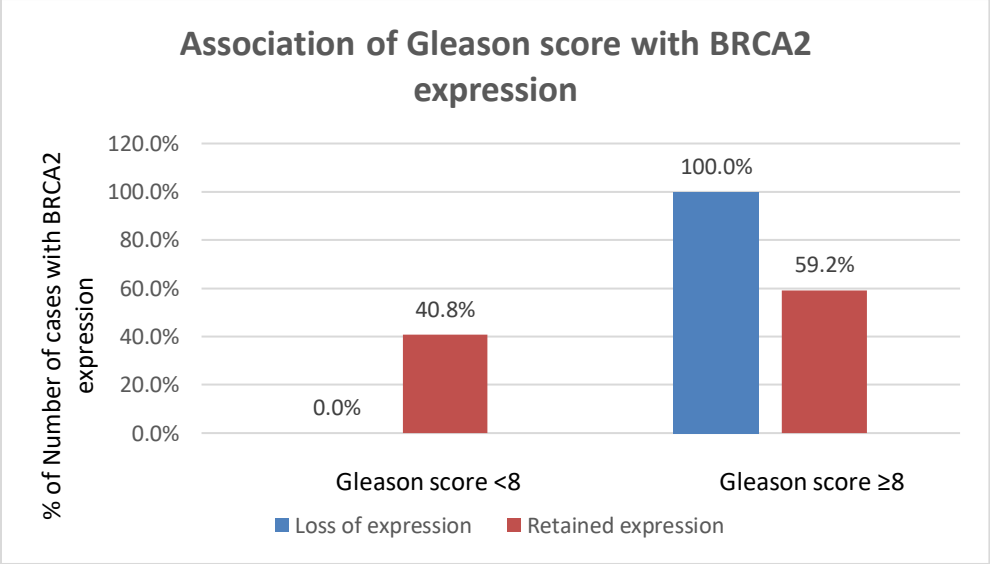


Figure 37.Component bar graph depicting association of Gleason score with BRCA2 expression

ASSOCIATION BETWEEN NEW CONTEMPORARY PROSTATE CANCER GRADING & BRCA1 EXPRESSION:

The Chi-square test is used to study the correlation between New contemporary prostate cancer grading and BRCA1 expression. 66.7% of the cases with loss of BRCA1 expression, only 37.5% of patients with retained expression showed Grade Group 5, indicating that patients with lost

expression had higher grade groups. However, the result was not found to have a statistically significant difference (p-value 0.77)

Figure 38.Component bar diagram showing the association of New contemporary prostate cancer grading with BRCA1 expression

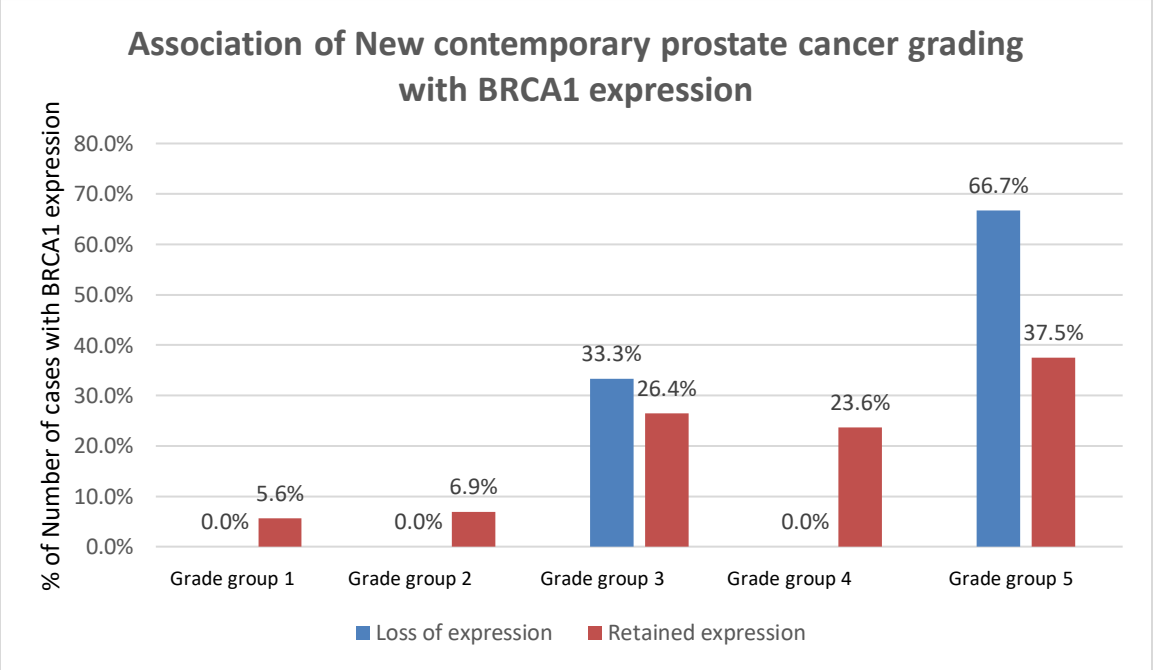


Table 21. Table depicting the association of New contemporary prostate cancer grading with BRCA1 expression

New contemporary prostate cancer grading	Loss of expression	Retained expression	p value
Grade group 1	0	4 (5.6%)	0.77
Grade group 2	0	5 (6.9%)	
Grade group 3	1 (33.3%)	19 (26.4%)	
Grade group 4	0	17 (23.6%)	
Grade group 5	2 (66.7%)	27 (37.5%)	

Chi square test used

ASSOCIATION BETWEEN NEW CONTEMPORARY PROSTATE CANCER GRADING & BRCA2 EXPRESSION:

The association between the New Contemporary Prostate Cancer Grading and BRCA2 Expression is investigated using the Chi-Square test. Of all the four cases with loss of BRCA2 expression, the majority of the cases (3 cases-75%) showed Grade group 5, and only one case (25%) showed grade group 4, demonstrating that cases with loss of expression of BRCA2 and higher grade groups (Grade groups 4&5). However, it was determined that there was no statistically significant difference in the result (p-value = 0.54).

Table 22.Table depicting association of New contemporary prostate cancer grading with BRCA2 expression

New contemporary prostate cancer grading	Loss of expression	Retained expression	p value
Grade group 1	0	4 (5.6%)	0.54
Grade group 2	0	5 (7%)	
Grade group 3	0	20 (28.2%)	
Grade group 4	1 (25%)	16 (22.5%)	
Grade group 5	3 (75%)	26 (36.6%)	

Chi square test used

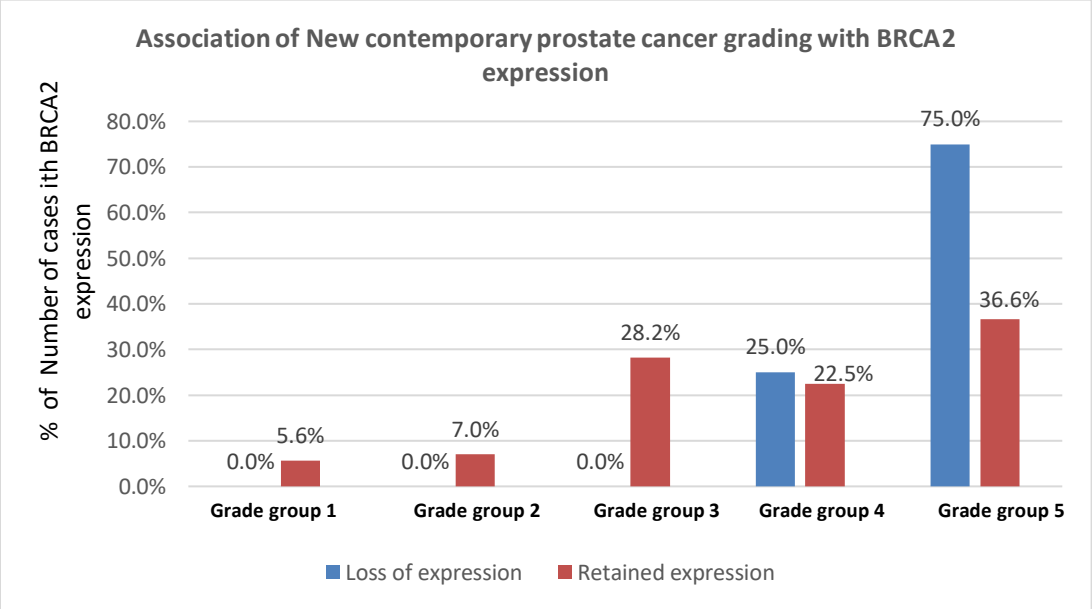


Figure 39.Component bar graph depicting the association of New contemporary prostate cancer grading with BRCA2 expression

ASSOCIATION BETWEEN TNM STAGING & BRCA1 EXPRESSION:

BRCA1 expression was compared with TNM staging in the below table. The results were not statistically not significant (p=0.07)

Table 23.Table depicting Association of TNM staging with BRCA1 expression

TNM stage	Loss of expression	Retained expression	p value
T2	0	41 (56.9%)	0.07
T3	3 (100%)	25 (34.7%)	
T4	0	6 (8.3%)	

Chi square test used

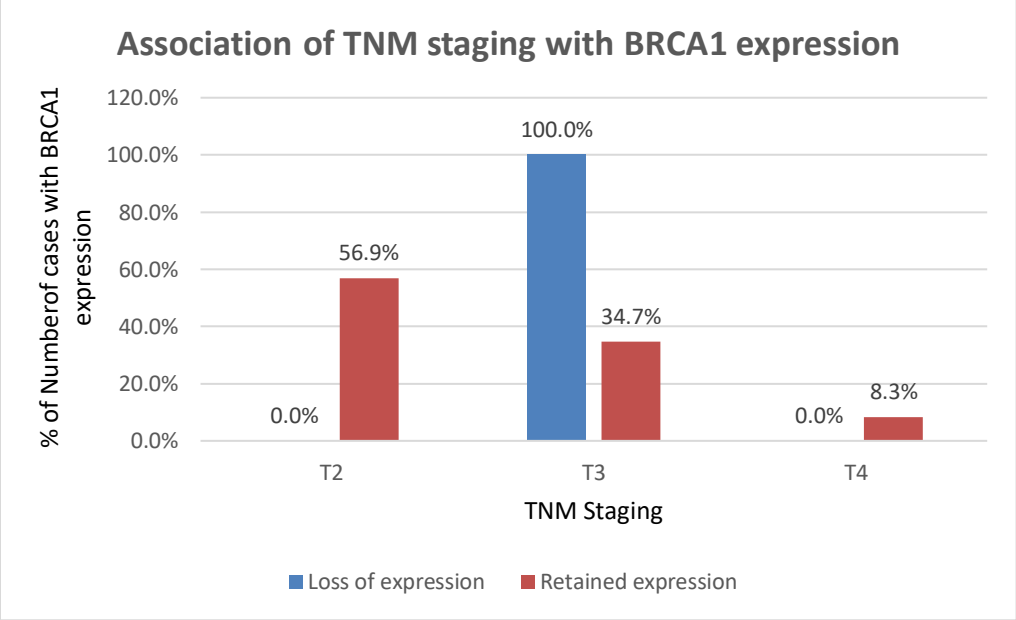


Figure 40.Component bar graph depicting the association of TNM staging with BRCA1 expression

ASSOCIATION BETWEEN TNM STAGING & BRCA2 EXPRESSION:

Although the majority (75%) of number of cases with loss of BRCA2 expression, show higher TNM staging (≥T3, T4) than non carriers with retained expression (43.6%) , p value was not found to be statistically significant (p=0.30)

Table 24. Table depicting association of TNM staging with BRCA2expression

TNM stage	Loss of expression	Retained expression	p value
T2	1 (25%)	40 (56.3%)	0.30
T3	2 (50%)	26 (36.6%)	
T4	1 (25%)	5 (7%)	

Chi square test used

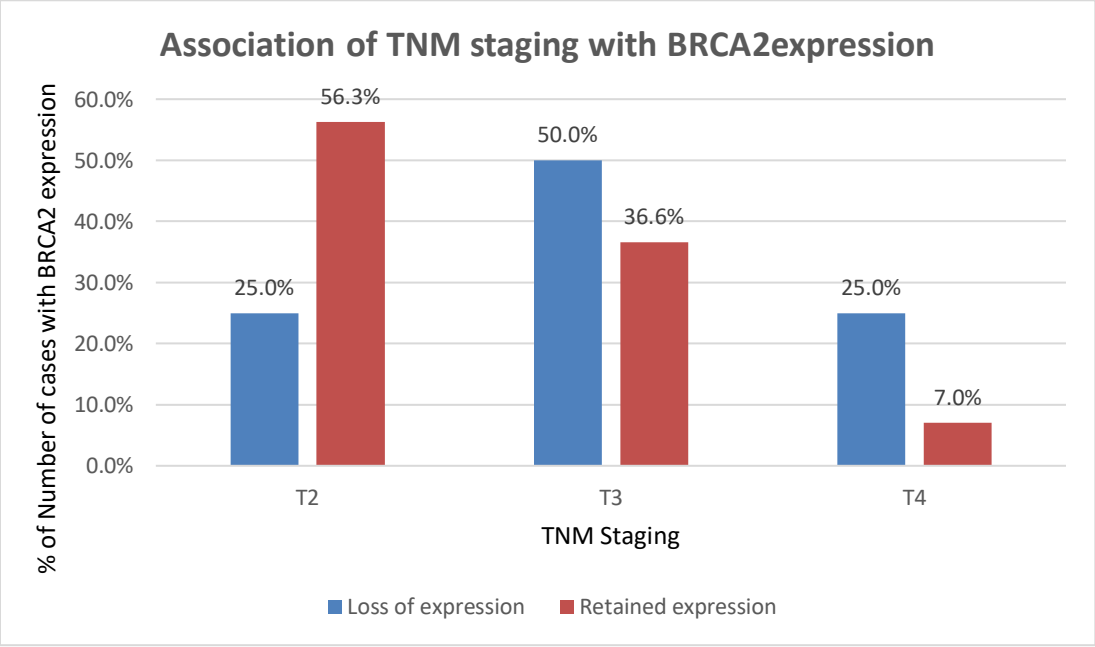


Figure 41.Component bar graph depicting the association between TNM staging & BRCA2 expression

ASSOCIATION BETWEEN SEMINAL VESICLE INVOLVEMENT & BRCA1 EXPRESSION:

The Fisher exact test determined the association between seminal vesicle involvement and BRCA1 expression. 66.7% of cases with loss of expression, whereas only 30.6% with retained BRCA1 expression show seminal vesicle involvement. The results were not found to be statistically significant (p-value:0.23)

Table25.Table depicting association of Seminal vesicle involvement with BRCA1 expression

Seminal vesicle involvement	Loss of expression	Retained expression	p value
Involved	2 (66.7%)	22 (30.6%)	0.23
Not involved	1 (33.3%)	50 (69.4%)	

Fisher exact test

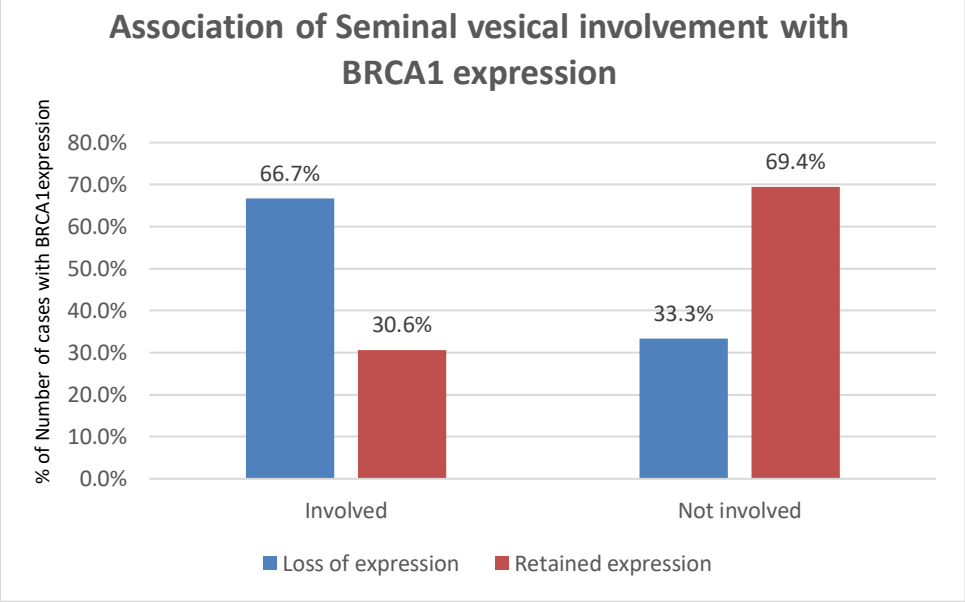


Figure 42.Component bar graph depicting the association between seminal vesicle involvement & BRCA1 expression

ASSOCIATION BETWEEN SEMINAL VESICLE INVOLVEMENT & BRCA2 EXPRESSION:

Although the majority of number of cases (75% of cases) with loss of BRCA2 expression, and just 29.6% of cases with retained BRCA2 expression show seminal vesicle involvement, indicating that seminal vesicle involvement is more common in patients with loss of BRCA2 expression. But the p-value was not found to be statistically significant (p=0.09)

Seminal vesicle involvement	Loss of expression	Retained expression	p value
Involved	3 (75%)	21 (29.6%)	0.09
Not involved	1 (25%)	50 (70.4%)	

Fisher exact test used

Table 26. Association of Seminal vesicle involvement with BRCA2 expression

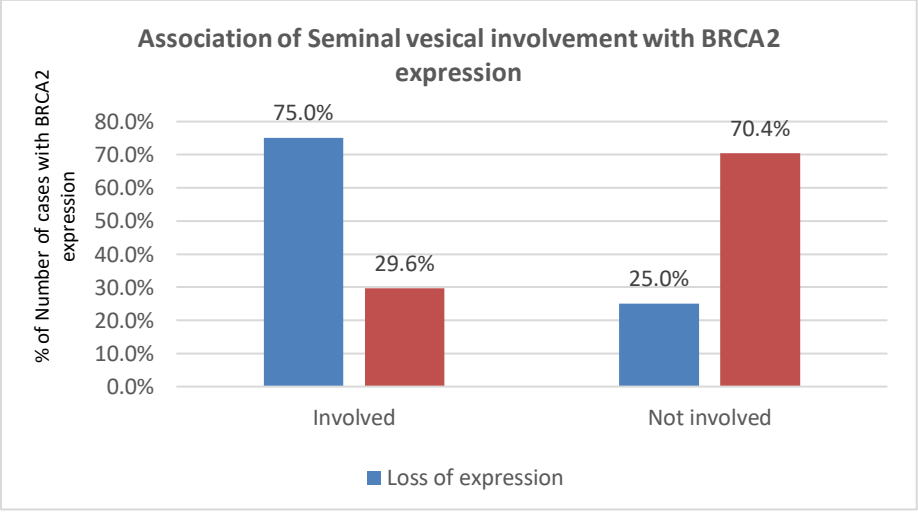


Figure 41.Component bar graph depicting the association between seminal vesicle involvement & BRCA2 expression

ASSOCIATION BETWEEN REGIONAL LYMPH NODE INVOLVEMENT & BRCA1 EXPRESSION

None of the cases show with lost BRCA1 expression show involvement of regional lymphnode and p value of 1.0 which is statistically insignificant.

Table 27.Association of Regional lymph node involvement with BRCA1 expression

Regional lymph node	Loss of expression	Retained expression	p value
Involved	0	8 (11.1%)	1.0
Not involved	3 (100%)	64 (88.9%)	

Fisher exact test used

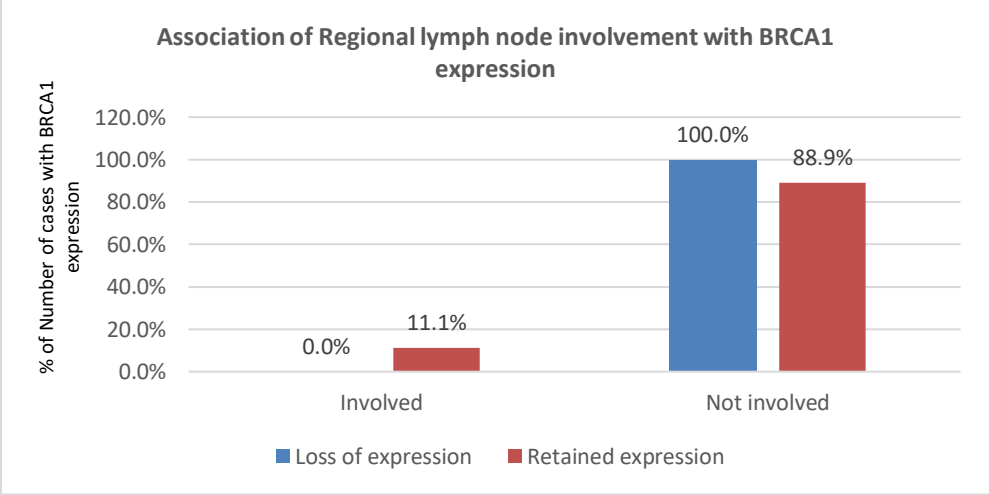


Figure 44.Component bar graph depicting the association between Regional lymph node involvement & BRCA1 expression

ASSOCIATION BETWEEN REGIONAL LYMPH NODE INVOLVEMENT & BRCA2 EXPRESSION

The association between the regional lymph node involvement and BRCA2 Expression is determined using the Fisher exact test. In total, 25% of cases with loss of BRCA2 expression, whereas in patients with retained BRCA2 expression, only 9.9% of cases show lymph node involvement. However, it was found that there was no statistically significant difference in the result (p-value = 0.36).

Table 28. Association of Regional lymph node involvement with BRCA2 expression

Regional lymph node	Loss of expression	Retained expression	p value
Involved	1 (25%)	7 (9.9%)	0.36
Not involved	3 (75%)	64 (90.1%)	

Fisher exact test used

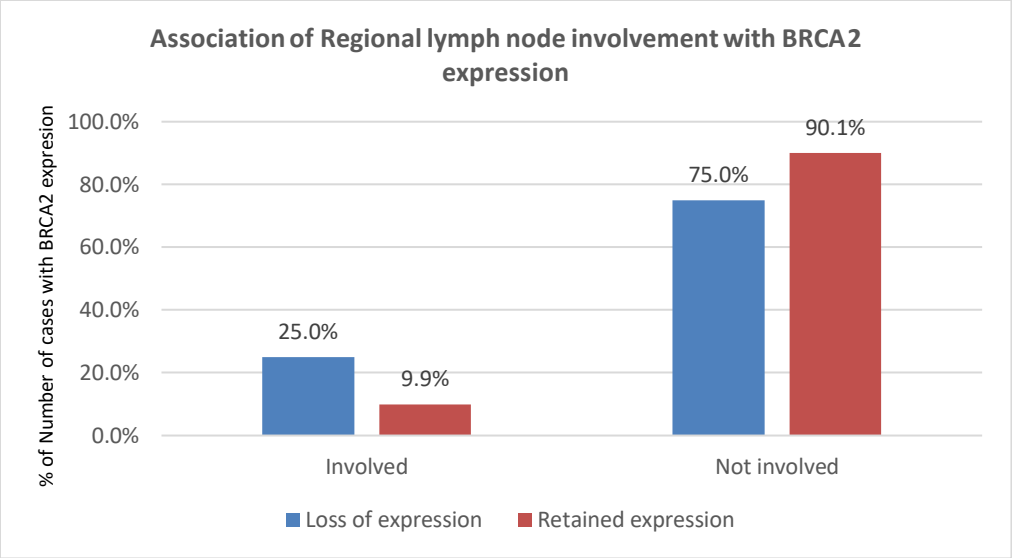


Figure 45. Component bar graph depicting the association between Regional lymph node involvement & BRCA2 expression

ASSOCIATION BETWEEN METASTASIS & BRCA1 EXPRESSION

None of the cases show with lost BRCA1 expression showed metastatic deposits and p value of 1.0 which is statistically insignificant.

Table 29. Association of Metastasis with BRCA1 expression

Metastasis	Loss of expression	Retained expression	p value
Present	0	7 (9.7%)	1.0
Absent	3 (100%)	65 (90.3%)	

Fisher exact test used

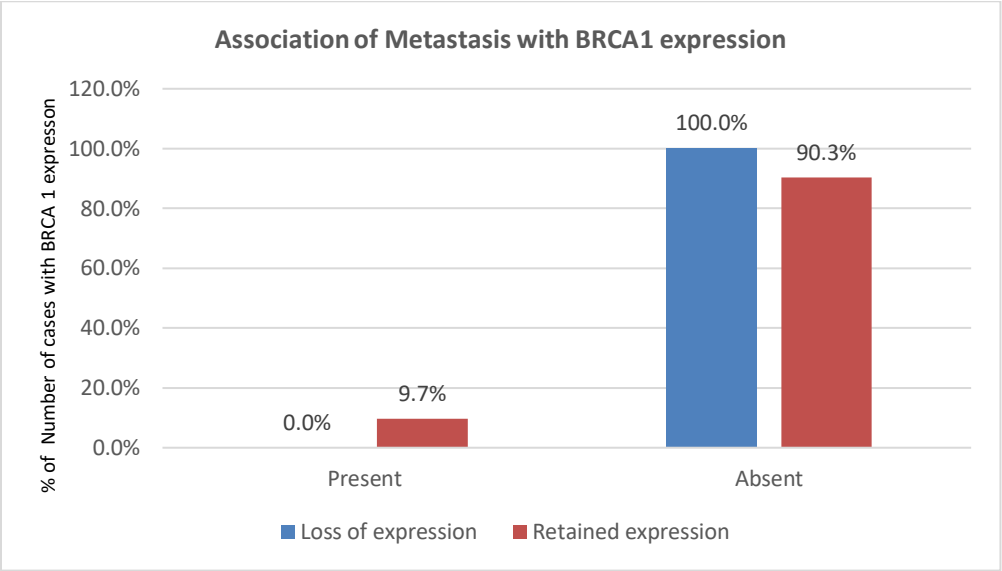


Figure 46.Component bar graph depicting the association between Metastasis & BRCA1 expression

ASSOCIATION BETWEEN METASTASIS & BRCA2 EXPRESSION

It was observed that 25% of cases with BRCA2 lost expression show metastatic deposits,and only 8.5% cases with retained BRCA2 expression showed the same. The results were not statistically significant (p value-0.33)

Table 30.Association of Metastasis with BRCA2 expression

Metastasis	Loss of expression	Retained expression	p value
Present	1 (25%)	6 (8.5%)	0.33
Absent	3 (75%)	65 (91.5%)	

Fisher exact test used

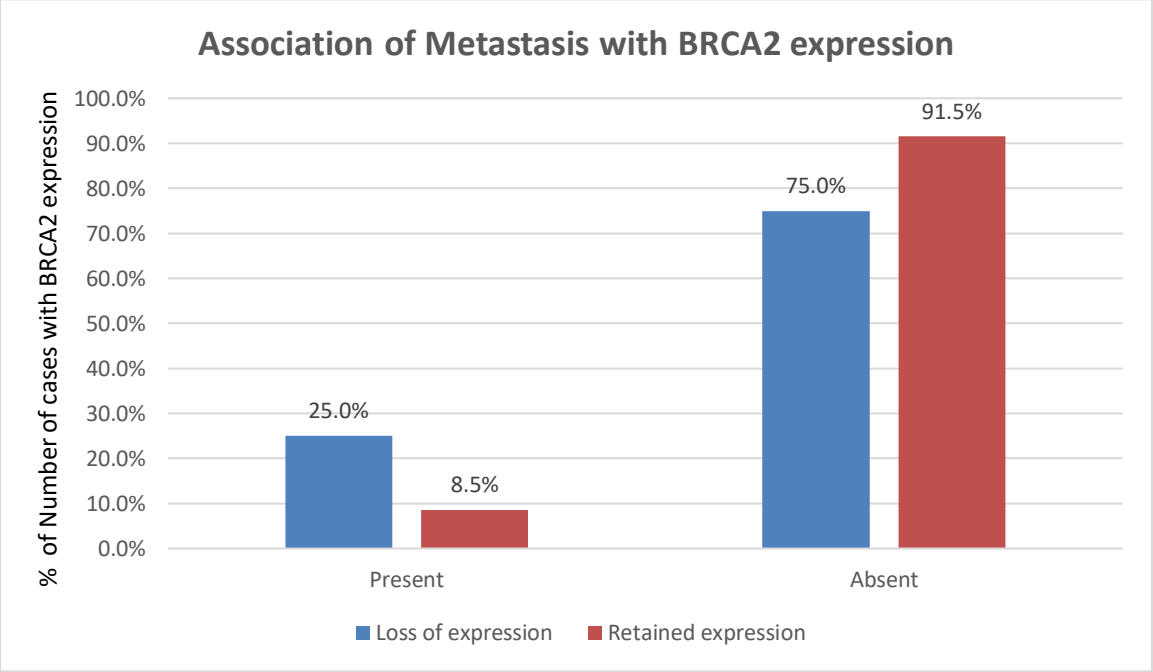


Figure 47. Component bar graph depicting the association between Metastasis & BRCA2 expression

ASSOCIATION BETWEEN Ki 67 & BRCA1 EXPRESSION

Of all the cases, 66.7% of cases with loss of BRCA1expression show and only 20.8% with retained BRCA1 expression show high proliferative index of Ki67. The results were not found to be statistically significant (pvalue-0.12)

Table 31.Table depicting association of Ki 67 with BRCA1 expression

Ki 67	Loss of expression	Retained expression	p value
High proliferative index	2 (66.7%)	15 (20.8%)	0.12
Low proliferative index	1 (33.3%)	57 (79.2%)	

Fisher exact test used

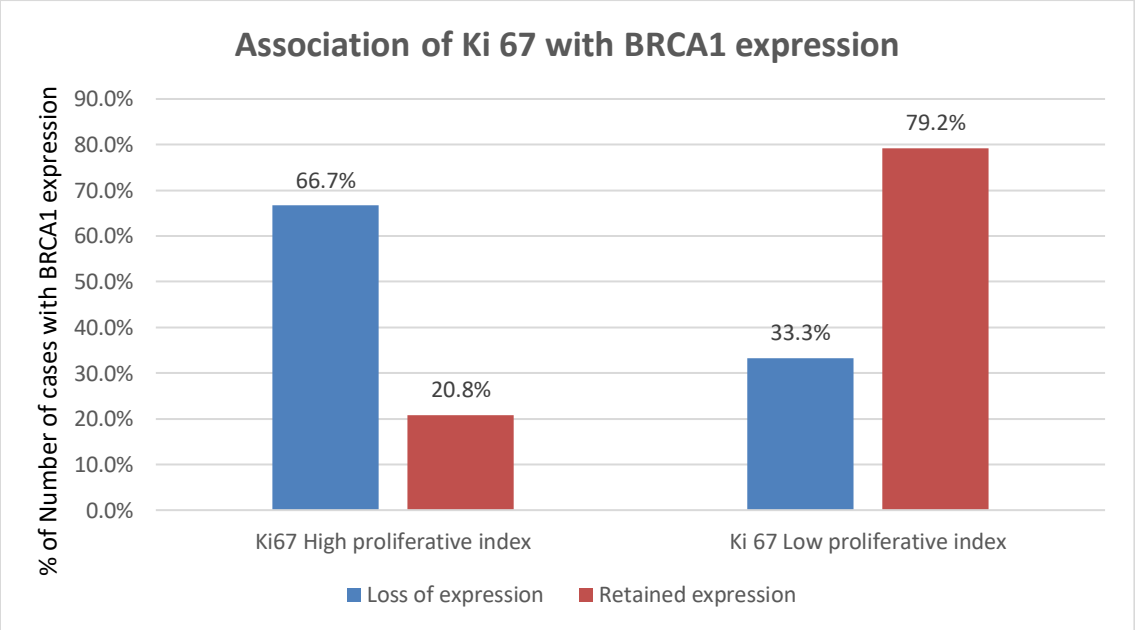


Figure 48.Component bar graph depicting the association between Ki67 & BRCA1 expression

ASSOCIATION BETWEEN Ki 67 & BRCA2 EXPRESSION

In subjects with loss of expression of BRCA2 all patients (100%) had high proliferative level of Ki67, while in subjects retained expression high proliferative level of Ki67 was seen in 18.3% patients. This difference was found statistically significant (p value <0.01).

Table 32.Table depicting association of Ki 67 with BRCA2 expression

Ki 67	Loss of expression	Retained expression	p value
High proliferative index	4 (100%)	13 (18.3%)	**<0.01**
Low proliferative index	0	58 (81.7%)	

Fisher exact test used

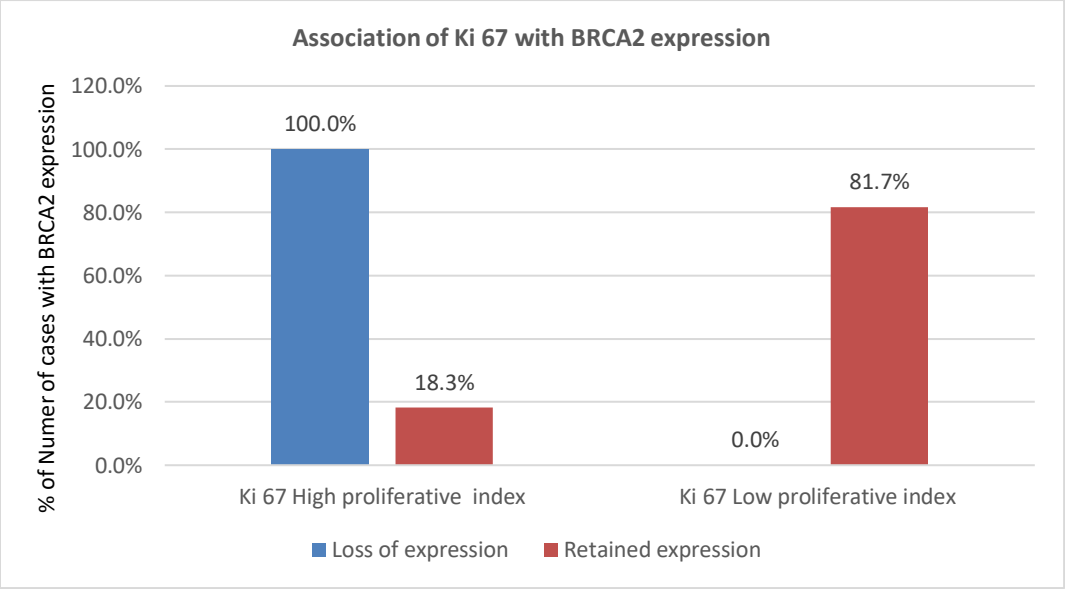


Figure 49.Component bar diagram showing association of Ki 67 with BRCA2 expression

ASSOCIATION BETWEEN Ki 67 & TNM STAGING

In subjects with high proliferative index of Ki67, T4 stage was significantly more common (17.6%) compared to subjects with low proliferative index (5.2%).

Table 33.Table depicting association between Ki67 and TNM staging

TNM staging	High proliferative index	Low proliferative index	p value
T2	5 (29.4%)	36 (62.1%)	**0.03**
T3	9 (52.9%)	19 (32.8%)	
T4	3 (17.6%)	3 (5.2%)	

Chi square test used

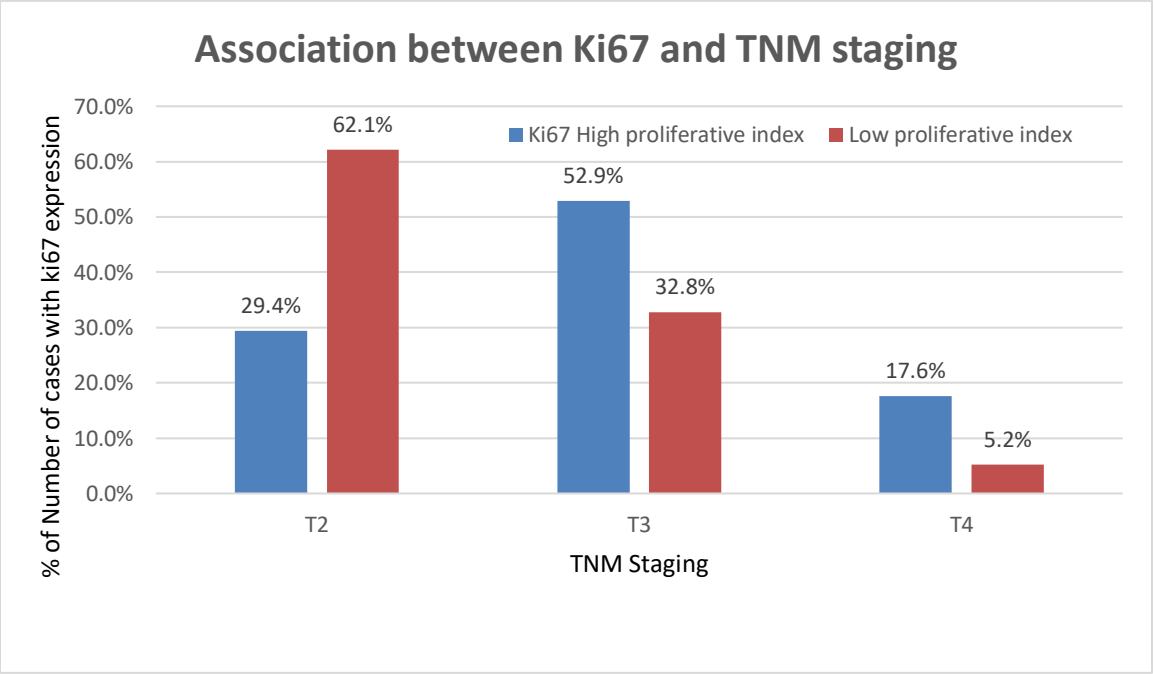


Figure 50.Component bar graph depicting the association between Ki67 & TNM staging

ASSOCIATION BETWEEN GLEASON SCORE & TNM STAGING

Chi square test used to determine association between Gleason score and TNM staging and results were found insignificant. (p value-0.12)

Table 34. Table depicting association between Gleason score and TNM staging

TNM staging	Gleason score <8	Gleason score ≥8	p value
T2	20 (69%)	21 (45.7%)	0.12
T3	8 (27.6%)	20 (43.5%)	
T4	1 (3.4%)	5 (10.8%)	

Chi square test used

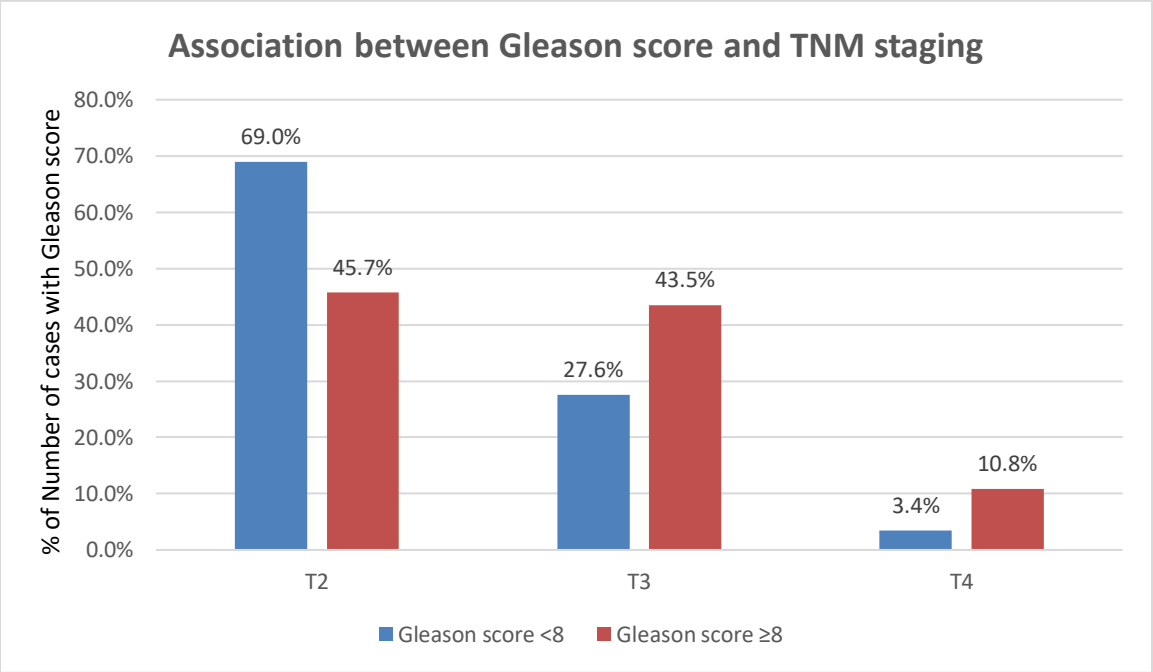


Figure 51.Component bar graph depicting the association between Gleason score & TNM staging

ASSOCIATION BETWEEN Ki 67 & BONE METASTASIS

Association between Ki67 & bone metastasis was determined using Fisher exact test. In subjects with high proliferative level of Ki67, bone metastasis was significantly more common (29.4%) compared to subjects with low proliferative level (3.4%).

Table 35.Table depicting the association between Ki67 and Bone metastasis

Bone metastasis	**Ki 67 High proliferative index**	**Ki 67 Low proliferative index**	**p value**
Present	5 (29.4%)	2 (3.4%)	**<0.01**
Absent	12 (70.6%)	56 (96.6%)	

Fisher exact test used

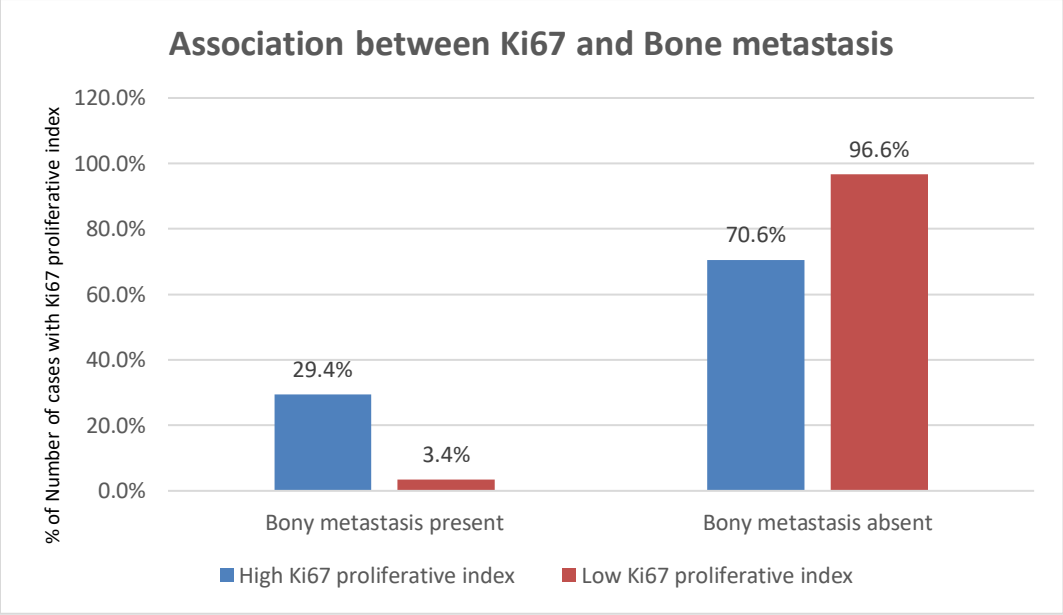


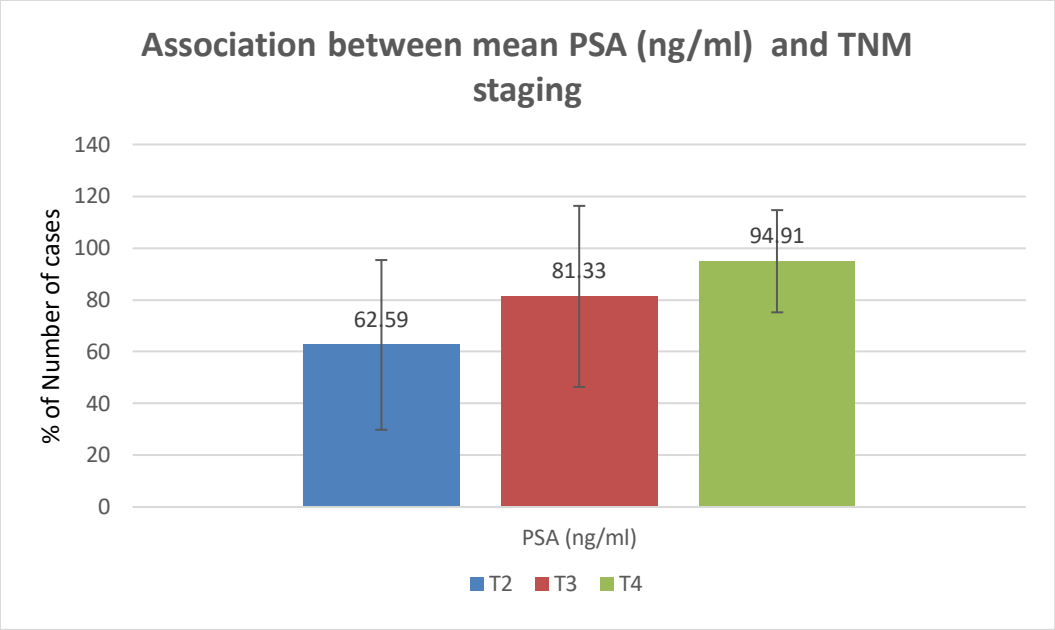
Figure 52.Component bar graph depicting the association between Ki67 & bony metastatsis

ASSOCIATION BETWEEN PSA and TNM STAGING:

Kruskal Wallis H test used to find association between PSA and TNM staging Mean Prostate specific antigen was 62.59±32.78 in T2 stage, 81.33±34.96 in T3 stage and 94.91±19.74 in T4 stage. With higher PSA level , the TNM stage was also higher. This association was found statistically significant (p value = 0.01).

Table 36. Table depicting association between PSA (ng/ml) and TNM staging

TNM stage	**Mean PSA (ng/ml)**	**SD**	**p value**
T2	62.59	32.78	**0.01**
T3	81.33	34.96	
T4	94.91	19.74	


Association between mean PSA (ng/ml) and TNM staging
140
120
100
80
60
40
20
0
% of Number of cases
62 59
81 33
94 91
PSA (ng/ml)
T2
T3
T4

DISCUSSION

The present study was conducted in the Department of Pathology and Lab Medicine, AIIMS. Patients who presented with complaints of lower urinary tract symptoms to the Department of Urology, AIIMS, were analysed. All the patients underwent surgical procedures like core biopsies, TURP and radical prostatectomy, which were received in the histopathology section of the Department of Pathology and Lab Medicine, AIIMS was extensively grossed, and microscopic examination was done and confirmed the diagnosis as Prostate adenocarcinoma.

During the period of January 2018 to June 2023, a feasible sample size of 75 cases with prostate carcinoma was included in the present study.

Table 37.Prevalence of BRCA mutations in prostate carcinoma in various studies :

Study/ Research	No: of Prostate cancer cases	Method used to detect BRCA Mutations	RESULT (% OF CASES WITH BRCA 1 MUTATIONS)	RESULT (% OF CASES WITH BRCA 2 MUTATIONS)
TCGA (91)	333		BRCA1 -*1%*	BRCA2 -*3%*
Pritchard et al (99)	692	Whole exome sequencing	BRCA1-0.9%	*BRCA2* - 5.3%
Robinson et al (100)	150		BRCA1-0.3%	BRCA2-13%
Nicolosi et al (94)	3607	Paired-end sequencing on an Illumina next-generation	BRCA1- 6.4%	BRCA2-24.3%

		sequencing platform		
Abida et al (101)	451	Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets sequencing assay	BRCA1-1%	BRCA2- 9%
Edwards et al(11)	263	PCR, Genome sequencing		BRCA2-2.3%
Ikonen et al (102)	548	Minisequencing, PCR		BRCA2-2.3%
Kirchoff et al(103)	251		BRCA1 -2%	BRCA2-3.1%
Trygvadottir et al(85)	527	PCR		BRCA2-3.1%
Agalliu et al(104)	979		BRCA1-3%	BRCA2-1.8%
Manguoglu et al (105)	50	Denaturing gradient gel electrophoresis and denaturing high performance liquid chromatography		BRCA2-2%
Gallagher et al (10)	832	Taqman (fluorogenic 5′ nuclease) assay & PCR	BRCA 1 -0.7%	BRCA2- 2.4%

Thorne et al(90)	148	PCR & Sanger sequencing		BRCA2 – 24.3%
Kote jarai et al (89)	1832	Multiplex fluorescence heteroduplex detection system		BRCA2 – 1%
Akbari et al (106)	1904			BRCA2 -1.4%
Maier et al(107)	474	PCR and sanger sequencing		BRCA2 -1.1%
Rong Na et al (108)	799	Whole exome sequencing and sanger sequencing		BRCA2 -1.9%
Amsi et al(109)	188	Immunohistochemistry	BRCA1- 26.1%	BRCA2-22.9%
Present study	75	Immunohistochemistry	BRCA1-4%	BRCA2-5.3%

The various studies done on the expression of BRCA mutations in prostate cancer cases are depicted in the table. Most previous studies detected BRCA1 and BRCA2 expression through molecular techniques like PCR, sangers sequencing and whole genome sequencing.

Amisi et al. included immunohistochemistry as a method of detection of BRCA1 and BRCA2 expressions done on 188 patients in which the majority of the population included are men of African descent, and resulted in a higher prevalence of BRCA1 and BRCA2 26.1% and 22.9% respectively(109). The present study also included immunohistochemistry as a method of detecting BRCA1 and BRCA2 expressions, and the prevalence was found to be 4% for BRCA1 and 5.3% for BRCA2 expression due to the small sample size (n=75) and Indian ethnicity. The BRCA2 prevalence in the current study was equal to a similar survey done by Pritchard et al., which is 5.3%, but that study comprised 692 cases of prostate cancer. (99)

To our knowledge, this is one of the largest studies in central India to date that has investigated the clinical characteristics of prostate cancer patients with and without BRCA mutation.

Many of the previous studies were performed on the population in Iceland, Ashkenazi Jewish men who are more prone to developing BRCA mutations than Indians included in our study.

The Mean age at diagnosis in prostate cancer cases in various studies :

Study/ Research	Mean age at diagnosis (in years)	Range (in years)
Edwards et al(11)	51	32-55
Ikonen et al (102)	66.1	50-82
Kirchoff et al (103)	68.4	65-71
Trygvadottir et al (85)	69	-
Agalliu et al (104)	65.8	43-87

Gallagher et al (10)	68	62-73
Thorne et al (90)	66.2	33–87
Castro et al(12)	58	42 -88
Akbari et al (106)	67	49–90
Maier et al (107)	61.1	42–80
Rong na et al (108)	62.0	57-68
Kammerer Jacquet et al(110)	70.93	66.77–73.61
Nicolosi et al(94)	60	-
Current study	65.8	44-90

Table 38.Depicting Mean age at diagnosis in prostate cancer cases in various studies

The youngest age group of 32-55 years are included in a survey done by Edwards et al., and in the remaining studies, the mean age at the time of diagnosis varied from 60-70 years.

In the present study, the age group of patients presented with adenocarcinoma prostate ranged between 44-90 years, and the mean age was 65.8 years. Agalliu et al. (104)conducted research with similar results concordant to the present study.

Correlation of age and BRCA expression:

In the current study, it is observed that the majority (66.7%) of patients with lost BRCA1 & BRCA2 expression were presented at a younger age(less than 60 years old); however, there is

no statistically significant difference was found between age and BRCA1 and BRCA2 expression ((p=0.57 for BRCA1 and p=0.27 for BRCA2)

In a study done by Gallagher et al. (88)on 832 cases with ages ranging between 65 and 73 years (mean age 68 years), the results were similar to the present study; there was no statistical difference in age at presentation between BRCA2 mutation carriers and noncarriers (P = 0.41) and between BRCA1 mutation carriers and noncarriers (P = 0.37).

According to a study done by Agalliu et al., the results concluded that early-onset prostate cancer (age at diagnosis < 55 years) had a higher prevalence of BRCA2 mutations in carriers (0.8% to 2.3%) compared to noncarriers.(104) The results in previous studies in the literature and the current study are similar, indicating that prostate cancer cases with BRCA mutations present at younger ages than noncarriers.

Contrary to these results, many patients with early onset prostate cancer (age at diagnosis <60 years) were analysed. However, no BRCA2 mutations were found in the study by Ikonen et al. (102)

Correlation of serum PSA and BRCA expression:

A study done by Castro et al.(12) found that median PSA was higher in BRCA2 (15.1 ng/ml) than in BRCA1 carriers (8.6 ng/ml). Similar results are found in our study. The mean PSA for BRCA2 carriers (103.82 ng/ml) was higher than in BRCA1 carriers (89.03 ng/ml)

Akbari et al. (106)and his colleagues found that the PSA concentration at diagnosis was higher among the cases with BRCA2 mutation than those without mutation (56.3 vs 13.3 ng ml 1, P<0.0001). Statistically significant results (103.82 vs 70.39 ng/ml, p=0.02) are also observed in the current study, implying the mean PSA of the cases with loss of BRCA2 expression (Carriers) was higher than cases with retained BRCA2 expression (noncarriers)

Maier et al. (107)discovered that the mean PSA of all BRCA mutation carriers was higher (38 ng/ml) as compared to 9.60 ng/ml of nonmutation cases, and we also observed similar higher PSA findings in the BRCA mutation carriers.

Association between PSA and TNM STAGING:

According to a study done by Zheng et al.,(111) PSA levels < 4 ng/ml have more favourable tumour characteristics, whereas PSA levels> 4 ng/ml had advanced TNM stage, and the results were found to be statistically significant ($p < 0.001$)

In the present study, the result concludes that with a higher PSA level, the TNM stage was also higher. This association was found statistically significant (p-value = 0.01). Both studies had similar observations.

Table 39. Table depicting ssociation between Gleason score & BRCA expression in various studies:

Study/Research	**% of BRCA2 carriers with Gleason score ≥8**	**% of BRCA2 Non carriers with Gleason score ≥8**	**P value**
Gallagher et al(10)	85%	57%	0.0002
Kote jarai et al(89)	63%	15%	<0.0001
Castro et al(12)	35%	15%	0.00003
Rong Na et al(108)	71%	31%	0.00009
Thorne et al (90)	65.8%	33%	0.04
Present study	100%	59.2%	0.15

In our current study, all the cases (100%) with BRCA2 mutation had a Gleason score of ≥8, whereas only 59.2% of BRCA2 noncarriers had a Gleason score of ≥8; the findings are almost similar to that of the investigation done by Gallagher et al . However the results in our study was statistically insignificant due to small sample size.

Agalliu et al**.** and his colleagues in their study found that a statistically significant association was observed for BRCA2

mutation carriers for prostate cancer cases with a Gleason score≥8.

Compared with previous studies, our observation found concordance with the observations made by Thorne et al., Kote Jarai et al., Castro et al., Rong Na et al., Trygvadotir et al. (108,85,90,89,12)

Association between New Contemporary Prostate Cancer Grading & BRCA1 expression:

According to a study done by **Trygvadotir et al.**, 84.0% of BRCA2 carriers had high grade groups G3 G4 at diagnosis, as compared with 52.7% of the noncarriers and none of the cases show Group grade 5 (85)

These findings are almost similar to observations in our current study in which most cases with loss of BRCA expression showed Grade group 5 with 66.7% of BRCA1 carriers and 75% of BRCA2 carriers, respectively. This study demonstrated that subjects with loss of expression of BRCA had higher grade groups than BRCA noncarriers, but results are insignificant, with a p-value of 0.54

Correlation between TNM staging & BRCA expression:

Several studies listed in the table showed that the patients with BRCA2 mutation (carriers) had a clinically aggressive disease with advanced stage (T3, T4) and worse outcomes. Even in our study, we got almost the same findings. However, the value is not statistically significant (p=0.30)

Study/Research	% of BRCA2 carriers with advanced T stage (≥T3-T3/T4)	% of BRCA2 Non carriers with advanced T stage (≥T3-T3/ T4)
Thorne et al (90)	44.8%	22.6%
Trygvadottir et al (85)	79.3%	38.6%
Castro et al(12)	37%	28%
Present study	75%	43.6%

Table 40. Comparing the correlation between TNM staging & BRCA expression in various studies

Also, in studies performed by Akbari et al. & Maier et al., 45.8% and 60% of BRCA2 carriers had an advanced stage- (T3 or T4), respectively.(106,107)

Association of BRCA expression and Metastasis:

In a study conducted by Trygvadottir et al., most (55.2%) of BRCA2 mutation carriers had metastatic disease at diagnosis, compared with 24.6% of noncarriers.

In the present study, 25% of BRCA2 mutation carriers had metastasis as compared with 8.5% of noncarriers, concluding that BRCA carriers had more metastatic disease compared to noncarriers because of the limited sample size included in the study (n=75 cases)

Association between Ki 67 and BRCA expression :

In the study by Byunn et al., a 10% cut-off value for the ki 67 proliferation index was revealed to have the best discriminating ability, which is taken as a cut-off in our present study.

Byunn et al. and his colleagues included 1,561 prostate cancer patients in their study and found that 13.8% of patients with high Ki-67 expression and 85.7% of patients with low Ki67 expression(97)

A similar observation was found in our present study; 22.7% of cases had been identified as cases with high Ki 67 proliferative index & 77.3% of cases identified as cases with low Ki 67 proliferative index

In the study done by Richardsen et al. in 535 cases, 61% had a low Ki67 expression, and 39% had a high Ki67 expression(112)

Kammerer Jacquet et al., in their study including 756 patients, found that 13% of patients had Ki67 >10 and 87% of patients showed ki67 <10. (110)

In the current study, while determining the association between BRCA1 expression and ki67, results were not found to be statistically significant (p-value: 0.12). However, in subjects with loss of expression of BRCA2 (carriers), all patients had high proliferative levels of Ki67. In contrast, in subjects with retained words (Noncarriers), a high proliferative level of Ki67 was seen in 18.3% of patients. This difference was found statistically significant (p-value <0.01).

Association between Ki 67 & TNM Staging

The current study observed that in subjects with a high proliferative index of Ki67, the T4 stage was significantly more common (17.6%) than in subjects with a low proliferative index (5.2%). (p-value = 0.03)

Similar findings were observed in a study done by Tretiakova et al.,(113) indicating that the Ki67 Proliferation index was significantly higher in patients with pathologic stages pT3/pT4 vs stage pT2 ($p<0.0001$)

Association between Ki 67 & Bone Metastasis

Association between Ki67 & bone metastasis was determined in the present study. In subjects with a high proliferative level of Ki67, bone metastasis was significantly more common (29.4%) compared to subjects with a low proliferative level (3.4%) (p-value <0.01)

A study conducted by Green et al. (114)suggested that Ki67 is a prognostic marker predictive of prostate cancer-specific survival and future metastasis (P=0.025)

SUMMARY

The study was conducted in the Department of Pathology and Laboratory Medicine, AIIMS Bhopal, to evaluate the Immunohistochemical expression of BRCA1 and BRCA2 mutations and their correlation with various clinicopathological variables to determine the prognosis in prostate carcinoma.

Although prostate cancer is one of the most common causes of cancer in males with a high mortality rate, there is minimal literature available in India about the topic of using Immunohistochemistry as a diagnostic modality to detect BRCA mutations, which plays a significant role in the carcinogenesis of prostate cancer.

An ambispective observational study included 75 diagnosed cases of Prostate Adenocarcinoma satisfying the inclusion criteria during the period of January 2018 to June 2023. All the study participants underwent IHC analysis of BRCA1 and BRCA2 and the association of BRCA expression with clinicopathological variables like Age, Serum PSA, Gleason score, New Contemporary grading cancer grading system., TNM staging and bone metastasis.

The prevalence of BRCA1 mutation was found to be 4%, and BRCA2 mutation was found to be 5.3% among the study participants. These mutations are considered to be sporadic rather than germline mutations. The age group of the study population varied from 44-91 years, with mean age calculated as 65 years. Majority of patients with lost BRCA1 and BRCA2 expression (carries)

belong to the young age group of less than 60 years, but the results are statistically insignificant. A statistically significant result (p-value = 0.02)was found when the serum PSA levels were

correlated with BRCA2 expression, suggesting that Mean Prostate specific antigen was higher in BRCA2 carriers compared to non carriers.

Both the Gleason score and New Contemporary grading cancer grading system showed statistically non-significant results due to limited sample size when they were compared with the association of BRCA1 and BRCA2 mutation. However, the current study showed that carriers of BRCA1/2 mutation had higher Gleason scores and grade groups when compared to BRCA 1/2 non carriers.

The TNM staging was also found to be in advanced stages (T3, T4) in cases with carriers of BRCA1/2 mutation compared to non-carriers. Still, the statistical difference was not found to be significant because of the small study sample size.

The majority of cases with BRCA1 mutation and entire cases of BRCA2 mutation showed a high Ki67 proliferative index, and the results were found statistically significant ($p < 0.01$) for association of Ki67 with BRCA2 mutation but not for BRCA1 mutation.

In this study, it was observed that bony metastasis was significantly more common (17.6%) in cases with a high proliferative index of Ki67 compared to patients with a low Ki67 proliferative index (p-0.03), indicating there is an association between Ki67 proliferative index and bony metastasis.

In addition to these, the current study also found an association between PSA and TNM staging. With a higher PSA level, the TNM stage was also higher. This association was found to have statistically significant results. (p-value -0.01).

CONCLUSION

The observations of the current study concluded that BRCA1 and BRCA2 mutation carriers presented at a younger age, had high Gleason core (≥8), higher Grade groups, advanced TNM staging (T3, T4), more probability of lymph node involvement and bony metastasis and clinically more aggressive disease with worse outcomes. So, this study signifies the importance of screening BRCA1 and BRCA2 mutations, especially for early onset prostate cancer patients, for predicting the prognosis and guiding the management strategies of these prostate cancer patients.

STRENGTHS OF STUDY:

1. There are several platforms like molecular testing (PCR, NGS) to study BRCA1 and BRCA2 expression, but Immunohistochemistry is a robust and readily available platform which allowed us to standardize this test as a modality.
2. The role of biomarkers like BRCA1 and BRCA2 in correlation with clinicopathological variables in Prostate adenocarcinoma have been documented in few studies in foreign literature, and there is very scant data about using IHC for detection of BRCA mutation in Indian literature to the best of our knowledge.
3. The study guided to bridge the gap in the present knowledge and led to a better understanding of Prostate cancer pathology.

LIMITATIONS OF STUDY

1. The study population was small; therefore, there is a possibility that the actual prevalence of BRCA mutations may be underrepresented.
2. The study included only the Indian population; other racial and ethnic groups, like African Americans, Iceland people, and Ashkenazi Jewish men, who are more prone to develop BRCA mutations, were not included.
3. The current study performed only immunohistochemical analysis, but comparison with other molecular testing methods for BRCA1 and BRCA2 mutations was not possible.
4. Long term follow-up of patients and survival analysis were not done in the present study.

FUTURE SCOPE/ RECOMMENDATIONS OF STUDY

1. Based on the observations of the current study, the authors recommend that BRCA1 and BRCA2 mutation carriers present at a younger age with aggressive clinical course and poor prognosis. Furthermore, the emerging role of BRCA1 and BRCA2 mutations underlines the growing significance of genetic counselling and the multidisciplinary approach in the management of prostate cancer patients.
2. These patients who inherited mutations in the BRCA genes respond well to PARP (Poly ADP-ribose polymerase) inhibitors and platinum-based chemotherapy. Additional research on other novel prostate carcinoma markers can be conducted in future to develop targeted therapy.

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PROFORMA (CASE RECORD FORM)

Patient ID:

Patient Name: Age: Sex:

Address:

CLINICAL DETAILS

Presenting complaints :

Clinical Examination :

Location of tumor :

INVESTIGATIONS :

Serum PSA (if done) :

BONE SCAN (if done) :

PROCEDURE :

HISTOPATHOLOGICAL DIAGNOSIS

Gleasons score

New contemporary prostate cancer grade:

Percentage of core in tumor

Lymphovascular invasion

Perineural invasion

IHC for diagnosis (P63, AMACR and tissue PSA):

TNM staging

PARTICIPANT INFORMATION SHEET

Title – "Immunohistochemical expression of BRCA mutations and its correlation with clinicopathological variables in Carcinoma Prostate"

Prostate adenocarcinoma is one of the most common cancers now a days . If left untreated, they may be associated with significant morbidity and mortality. In the present study we are trying to correlate the IHC expression of BRCA 1 and BRCA2 and correlation with clinicopathological variables in prostate adenocarcinoma so that appropriate and accurate treatment can be started at the earliest and there by having a better prognosis. If shown to be significant, these correlations can be used for diagnosing and treating other patients with the same conditions as yours.

This study is being conducted by Dr Dega Vamseekrishna, PG resident at department of Pathology and Lab Medicine under the guidance of Dr Hemalata Panwar, Associate professor, Department of Pathology and Lab Medicine, Dr Ashwani Tandon, Professor, Department of Pathology and Lab Medicine, Dr Ujjawal Khurana, Associate professor, Department of Pathology and Lab Medicine, Dr Jai Kumar Chaurasia, Associate professor, Pathology and Lab Medicine, Dr Kumar Madhavan Associate professor, Department of Urology , AIIMS Bhopal

You are invited to participate in the above mentioned study which will be conducted at AIIMS, Bhopal between the time duration of January 2022 to June 2023. The decision to either participate or not depends entirely on you and it would not affect your treatment or follow up now or in the future. Please read the information carefully before giving your consent. In case the participant still has any inquiries, they can contact me at +918639000429 anytime for the clarifications.

1. Why you are being invited to participate in the study?

You are being invited as you fulfill the inclusion criteria of this study design. Approximately 75 people diagnosed with the disease and fulfilling the inclusion criteria are expected to participate in the study which shall be conducted at AIIMS, Bhopal.

2. Do I have to agree to being in the study?

Your participation in the study is completely voluntary. You can agree to participate or decline the invitation. In either situation, your treatment and follow up will not be affected. If you agree to participate in the study, you will have to fill a consent form and sign it. If at any time during the study you want to withdraw your participation, you are completely free to do so.

3. Why is this research being done?

The research is being done to assess the immunohistochemical expression of BRCA 1 and BRCA2 in prostate adenocarcinoma and its correlation with clinicopathological variables which will help in prognosis and treatment therapy of the patients decreasing morbidity and mortality

4.What will happen if I agree to participate in the study?

If you agree to participate in this study, you will have to sign a consent form allowing the researcher to use IHC markers on tissue sample and you will be asked some questions.

5.How long will I be required to take part in the study?

As this is a one-time study, so your participation over a length of time is required. However, you are free to deny participation in the study at any time during the study without having to give any reason.

6. Does this study involve any risks on my part?

No, this study does not involve any invasive tests on your person per se.

7.Are there any benefits for me to be taking part in the study?

No financial benefits shall be given for your participation in the study.

8. Are there any costs to be involved in the study?

No, you would not be asked to pay for any of the tests we perform in the study.

9. Will my identity be kept confidential?

Yes, you may rest assure that your identity will only be known by the above mentioned researchers. Any kind of identifiable information regarding you will not be revealed to any third party unless you give your permission for doing so or if required by the law.

10.What will be done to the information gathered during the study?

The information collected during the study will be used for medical reference and research purpose only. The result of the study may be published in medical journal or be shared with another research entity, but the identity of the participants will not be revealed. Once you have

read this information sheet, the researcher will answer any doubts that you might have. If you wish to participate in the proposed study, kindly sign the attached consent form.

प्रतिभागी सूचना पत्र

शीर्षक - " बीआरसीए म्यूटेशन की इम्यूनोहिस्टोकेमिकल अभिव्यक्ति और कार्सिनोमा प्रोस्टेट में इसके रोगसूचक महत्व"

प्रोस्टेट एडेनोकारिनोमा आजकल सबसे आम कैंसर में से एक है। यदि अनुपचारित छोड़ दिया जाता है, तो वे महत्वपूर्ण रुग्णता और मृत्यु दर से जुड़े हो सकते हैं। वर्तमान अध्ययन में हम बीआरसीए 1 और बीआरसीए 2 की आईएचसी अभिव्यक्ति और प्रोस्टेट एडेनोकार्सिनोमा में क्लिनिकोपैथोलॉजिकल चर के साथ सहसंबंध को सहसंबंधित करने का प्रयास कर रहे हैं ताकि बेहतर निदान होने पर जल्द से जल्द उचित और सटीक उपचार शुरू किया जा सके। यदि महत्वपूर्ण दिखाया गया है, तो इन सहसंबंधों का उपयोग अन्य रोगियों के निदान और उपचार के लिए किया जा सकता है, जिनके साथ आपकी स्थिति समान है। यह अध्ययन पैथोलॉजी एवं लैब मेडिसिन विभाग में पीजी रेजिडेंट डॉ. देगा वामसीकृष्णा द्वारा डॉ हेमलता पंवार, असिस्टेंट प्रोफेसर, पैथोलॉजी एंड लैब मेडिसिन विभाग, डॉ अश्विनी टंडन, एडिशनल प्रोफेसर, पैथोलॉजी एंड लैब मेडिसिन विभाग के मार्गदर्शन में किया जा रहा है. , डॉ दिनेश उज्जवल खुराना, सहायक प्रोफेसर, पैथोलॉजी और लैब मेडिसिन विभाग, डॉ जय कुमार चौरसिया, सहायक प्रोफेसर, पैथोलॉजी और लैब मेडिसिन, डॉ कुमार माधवन यूरोलॉजी विभाग, एम्स भोपाल

आपको उपर्युक्त अध्ययन में भाग लेने के लिए आमंत्रित किया जाता है जो जनवरी 2022 से जून 2023 की अवधि के बीच एम्स, भोपाल में आयोजित किया जाएगा। भाग लेने या न करने का निर्णय पूरी तरह से आप पर निर्भर करता है और यह आपके उपचार या अनुवर्ती कार्रवाई को प्रभावित नहीं करेगा। अभी या भविष्य में। कृपया अपनी सहमति देने से पहले जानकारी को ध्यान से पढ़ें। यदि प्रतिभागी के पास अभी भी कोई पूछताछ है, तो वे स्पष्टीकरण के लिए किसी भी समय +918639000429 पर मुझसे संपर्क कर सकते हैं।

1. आपको अध्ययन में भाग लेने के लिए क्यों आमंत्रित किया जा रहा है?
आपको आमंत्रित किया जा रहा है क्योंकि आप इस अध्ययन डिजाइन के समावेशन मानदंड को पूरा करते हैं। एम्स, भोपाल में आयोजित किए जाने वाले अध्ययन में बीमारी से पीड़ित और समावेशन मानदंडों को पूरा करने वाले लगभग 75 लोगों के भाग लेने की उम्मीद है।

2. क्या मुझे अध्ययन में शामिल होने के लिए सहमत होना होगा?
अध्ययन में आपकी भागीदारी पूरी तरह से स्वैच्छिक है। आप आमंत्रण में भाग लेने या अस्वीकार करने के लिए सहमत हो सकते हैं। किसी भी स्थिति में, आपका उपचार और अनुवर्ती कार्रवाई प्रभावित नहीं होगी। यदि आप अध्ययन में भाग लेने के लिए सहमत हैं, तो आपको एक सहमति फॉर्म भरना होगा और उस पर हस्ताक्षर करना होगा। यदि आप अध्ययन के दौरान किसी भी समय अपनी भागीदारी वापस लेना चाहते हैं, तो आप ऐसा करने के लिए पूरी तरह से स्वतंत्र हैं।

3. यह शोध क्यों किया जा रहा है?
प्रोस्टेट एडेनोकार्सिनोमा में बीआरसीए 1 और बीआरसीए 2 की इम्यूनोहिस्टोकेमिकल अभिव्यक्ति का आकलन करने के लिए शोध किया जा रहा है और क्लिनिकोपैथोलॉजिकल चर के साथ इसका संबंध है जो रोगियों की रुग्णता और मृत्यु दर को कम करने के लिए रोग का निदान और उपचार चिकित्सा में मदद करेगा।

4.यदि मैं अध्ययन में भाग लेने के लिए सहमत हूं तो क्या होगा?
यदि आप इस अध्ययन में भाग लेने के लिए सहमत हैं, तो आपको एक सहमति फॉर्म पर हस्ताक्षर करना होगा, जिसमें शोधकर्ता को ऊतक के नमूने पर IHC मार्करों का उपयोग करने की अनुमति होगी और आपसे कुछ प्रश्न पूछे जाएंगे।

5. मुझे अध्ययन में भाग लेने के लिए कितने समय की आवश्यकता होगी?
चूंकि यह एक बार का अध्ययन है, इसलिए लंबे समय तक आपकी भागीदारी आवश्यक है। हालांकि, आप बिना कोई कारण बताए अध्ययन के दौरान किसी भी समय अध्ययन में भाग लेने से इनकार करने के लिए स्वतंत्र हैं।

6. क्या इस अध्ययन में मेरी ओर से कोई जोखिम शामिल है?
नहीं, इस अध्ययन में आपके व्यक्ति पर कोई भी आक्रामक परीक्षण शामिल नहीं है।

7. क्या इस अध्ययन में भाग लेने से मुझे कोई लाभ होगा?
अध्ययन में आपकी भागीदारी के लिए कोई वित्तीय लाभ नहीं दिया जाएगा।

8. क्या अध्ययन में शामिल होने की कोई लागत है?
नहीं, आपको अध्ययन में किए जाने वाले किसी भी परीक्षण के लिए भुगतान करने के लिए नहीं कहा जाएगा।

9. क्या मेरी पहचान गोपनीय रखी जाएगी?
हां, आप निश्चिंत हो सकते हैं कि आपकी पहचान केवल उपर्युक्त द्वारा ही जानी जाएगी शोधकर्ताओं। आपके बारे में किसी भी प्रकार की पहचान योग्य जानकारी प्रकट नहीं की जाएगी कोई भी तीसरा पक्ष जब तक कि आप ऐसा करने के लिए अपनी अनुमति नहीं देते या यदि कानून द्वारा आवश्यक हो।

10.अध्ययन के दौरान एकत्रित की गई जानकारी का क्या किया जाएगा?
अध्ययन के दौरान एकत्र की गई जानकारी का उपयोग चिकित्सा संदर्भ और अनुसंधान उद्देश्य केवल

के लिए किया जाएगा। अध्ययन का परिणाम मेडिकल जर्नल में प्रकाशित किया जा सकता है या किसी अन्य शोध संस्था के साथ साझा किया जा सकता है, लेकिन प्रतिभागियों की पहचान का खुलासा नहीं किया

जाएगा। एक बार जब आप इस सूचना पत्र को पढ़ लेते हैं, तो शोधकर्ता आपके किसी भी संदेह का उत्तर देगा। यदि आप प्रस्तावित अध्ययन में भाग लेना चाहते हैं, तो कृपया संलग्न सहमति फॉर्म पर हस्ताक्षर करें।

INFORMED CONSENT FORM

Study title: "Immunohistochemical expression of BRCA mutations and its correlation with clinicopathological variables in Carcinoma Prostate"

Subject's name: Age/Sex:

S/O / D/O: Date:

Address:

I hereby declare that

1. I have been thoroughly explained about the study in my vernacular language by the researcher and all of my queries have been answered by the researcher.

2. I fulfill the criteria required for participation in this study.

3. I have understood the fact that my participation in the study is completely voluntary

and my decision would not affect treatment modality, at AIIMS Bhopal, in any way.

4. I have understood that I can withdraw my participation at any time during the study without giving any reasons.

5. I understand that I am not expected to pay for the costs of any of the special tests that the study involves.

6. I also understand that I shall not be given any perks or paid any money for my participation in this study.

7. I understand that my identity will be kept confidential and only the researchers involved with the study directly will be aware of my personal particulars.

8. I allow the researcher(s) to assess my clinical history and records required for the study.

9. I declare that all the information I provide to the researcher(s) is true to the best of my knowledge.

Signature of participant: Signature of witness:

Name of participant: Name of witness:

Thumb impression: Thumb impression of witness:

Signature of researcher:

Name of the researcher:

Date:

Place: Bhopal

सूचित सहमति प्रपत्र

अध्ययन शीर्षक: " बीआरसीए म्यूटेशन की इम्यूनोहिस्टोकेमिकल अभिव्यक्ति और कार्सिनोमा प्रोस्टेट में इसके रोगसूचक महत्"

विषय का नाम: आयु/लिंग:

एस/ओ/डी/ओ: दिनांक:

पता:

मैं एतद्द्वारा घोषणा करता हूं कि

1. मुझे मेरी स्थानीय भाषा में अध्ययन के बारे में पूरी तरह से समझाया गया है
शोधकर्ता और मेरे सभी प्रश्नों का उत्तर शोधकर्ता द्वारा दिया गया है।

2. मैं इस अध्ययन में भाग लेने के लिए आवश्यक मानदंडों को पूरा करता हूं।

3. मैं इस तथ्य को समझ गया हूं कि अध्ययन में मेरी भागीदारी पूरी तरह से स्वैच्छिक है
और मेरे निर्णय से एम्स भोपाल में किसी भी तरह से इलाज के तौर-तरीकों पर कोई असर नहीं पड़ेगा।

4. मैं समझ गया हूँ कि मैं अध्ययन के दौरान किसी भी समय अपनी भागीदारी वापस ले सकता हूँ
बिना कोई कारण बताए।
5. मैं समझता/समझती हूं कि मुझसे किसी विशेष परीक्षण की लागत का भुगतान करने की अपेक्षा नहीं की जाती है

जिसमें अध्ययन शामिल है।

6. मैं यह भी समझता/समझती हूं कि मेरे . के लिए मुझे कोई अनुलाभ या भुगतान नहीं किया जाएगा
इस अध्ययन में भागीदारी।

7. मैं समझता हूं कि मेरी पहचान गोपनीय रखी जाएगी और केवल शोधकर्ता
सीधे अध्ययन से जुड़े लोग मेरे व्यक्तिगत विवरणों से अवगत होंगे।

8. मैं शोधकर्ता(ओं) को अपने नैदानिक इतिहास और इसके लिए आवश्यक रिकॉर्ड का आकलन करने की अनुमति देता हूं
पढाई।

9. मैं घोषणा करता/करती हूं कि मेरे द्वारा शोधकर्ता(ओं) को प्रदान की गई सभी जानकारी सर्वोत्तम के लिए सही है
मेरा ज्ञान।
प्रतिभागी के हस्ताक्षर: गवाह के हस्ताक्षर:

प्रतिभागी का नाम: गवाह का नाम:

अंगूठे का निशान: साक्षी के अंगूठे का निशान:

शोधकर्ता के हस्ताक्षर:

शोधकर्ता का नाम:

दिनांक:

जगह: भोपाल

MASTER CHART

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	NAME	UHID	LAB NO	AGE	PSA(ng/ml)	SPECIMEN	GLEASON SCORE	IHC PD-	QUANTITATION OF TUMOF LVI	PNI	TNM STAGING	SENTINAL VESICLE	REGIONAL LYMPH NOD	METASTASIS	BRCA1 EXPRESSION (NUCLEAR STAINING)	BRCA2 EXPRESSION (CYTOPLASMIC+NUCLEAR STAINING)	KI67 (Low/High Proliferative index)
2	SANTOSHDUBEY	20170048101	5504-18	59	102.5	COREBIOPSY	4+3=7	Grade group 5	90%: NOTIDENTIFIED	NOTIDENTIFIED	pT3bN0Mx	NOTINVOLVED	NOTINVOLVED	NOTIDENTIFIED	Retinoid expression (Wild type Man carrier, Retinoid expression (Cytoplasmic+Nuclear staining)- Wild type/